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(54) Title: MULTIVALENT AND MULTISPECIFIC ANTIGEN-BINDING PROTEIN

(57) Abstract

A multivalent antigen-binding protein comprises a first polypeptide comprising, in series, three or more variable domains of an antibody heavy chain and a second polypeptide comprising, in series, three of more variable domains of an antibody light chain, said first and second polypeptides being linked by association of the respective heavy chain and light chain variable domains, each associated variable domain pair forming an antigen binding site. Methods for their production and uses thereof, in particular for therapeutic and diagnostic applications, are disclosed.

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MULTIVALENT AND MULTISPECIFIC ANTIGEN-BINDING PROTEIN

FIELD OF THE INVENTION

The present invention relates to multivalent and multispecific antigen binding proteins, methods for their production and uses thereof. In particular, the invention relates to binding proteins comprising polypeptides which associate to form multivalent or multispecific multimers.

BACKGROUND OF THE INVENTION

Antibodies are protein molecules having a structure based on a unit comprising four polypeptides, two identical heavy chains and two identical light chains, which are covalently linked together by disulphide bonds. Each of these chains is folded in discrete domains. The C-terminal regions of both heavy and light chains are conserved in sequence and are called the constant regions, comprising one or more so-called C-domains. The N-terminal regions of the heavy and light chains, also known as V-domains, are variable in sequence and determine the specificity of the antibody. The regions in the variable domains of the light and heavy chains (V_L and V_H respectively) responsible for antigen binding activity are known as the hypervariable or complementarity determining regions (CDR). Natural antibodies have at least two identical antigen-binding sites defined by the association of the heavy and light chain variable regions.

It is known that proteolytic digestion of an antibody can lead to the production of antibody fragments. Such fragments, or portions, of the whole antibody can exhibit antigen binding activity. An example of a binding fragment is an F_{ab} fragment which comprises a light chain

associated with the V_H and C_{H1} domains of a heavy chain. The bivalent $F(ab')_2$ fragment comprises two such F_{ab} fragments connected together via the hinge region, giving two antigen binding sites. F_v fragments, consisting only of the V-domains of the heavy and light chains associated with each other may also be obtained. These F_v fragments are monovalent for antigen binding. Smaller fragments such as individual V-domains (domain antibodies or dABs, Ward et al Nature, 341, 544 (1989) and individual CDR's (Williams et al, Proc. Natl. Acad. Sci, USA, 86, 5537 (1989)) have also been shown to retain the binding characteristics of the parent antibody although generally most naturally occurring antibodies need both a V_H and V_L to retain full immunoreactivity.

Antibody fragments comprising V_H and V_L domains associated together to have antigen binding activity have also been described. The single chain F_v fragment (scFv) comprises a V_H domain linked to a V_L domain by a flexible polypeptide linker such that the domains can associate to form an antigen binding site (see, for example, EP-B-0281604, Enzon Labs Inc).

Microbial expression systems for producing active antibody fragments are known in the literature. The production of Fab in various hosts such as *E.coli*. (Better et al, Science, 240, 104, (1988)), yeast (Horwitz et al, Proc. Natl. Acad. Sci, US4, 85, 8678 (1988)) and the filamentous fungus *Trichoderma reesei* (Nyyssönen et al, Bio/Technology, 11, 591 (1993)) have previously been described, for example. It is also known that plants can be used as hosts for the production of SCFv fragments (Owen et al, Bio/Technology, 10, 790 (1992)) as well as whole antibodies.

An advantage of using antibody fragments rather than whole antibodies in diagnosis and therapy lies in their

smaller size. They are likely to be less immunogenic than whole antibodies and more able to penetrate tissue. A disadvantage associated with the use of fragments such as the F_{ab}, F_v, and S_eF_v antibody fragments described above, however is that they have only one binding site for antigen binding as compared to the two or more sites contained in the whole antibody, preventing polyvalent binding to the antigen and hence leading to reduced avidity.

In an attempt to overcome this problem, attention has been directed to providing multivalent antigen binding proteins, that is binding proteins having more than one antigen binding site. In addition, there has been interest in producing antigen-binding proteins having multiple specificities capable of binding to different antigenic determinants and containing antigen binding domains derived from different sources. Antigen-binding proteins having distinct binding specificities may be useful, for example, in targeting effector cells to target cells by virtue of the specific binding of the different binding domains. By way of illustration, a bispecific antigen binding protein having specificity for both tumour cells and cytotoxic drugs may be used to target specifically cytotoxic drug to tumour cell in an efficient manner. By avoiding the need for chemical modification, adverse immune responses may be avoided.

Hitherto, the potential application of multivalent and multispecific antigen binding proteins have been hindered by the difficulties in generating and purifying such molecules.

Recombinant antigen-binding proteins having two binding sites may be prepared by methods such as chemical cross-linking of cysteine residues, either through cysteine residues introduced at the C-terminus of the V_H of an F_v

(Cumber et al, J.Immunol., 149, 120 (1992)), through the hinge cysteine residues in F_{ab} to generate $(Fab')_2$ (Carter et al, Bio/Tech., 10, 163 (1992)) or at the C-terminus of the V_L of an scFv (Pack and Plückthun, Biochemistry, 31, 1579 (1992)). Alternatively, the production of bivalent and bispecific antibody fragments based on the inclusion of F_{ab} fragments of C-terminal peptide sequences which promote dimerisation has been described. (Kostelny et al, J.Immunol., 148, 1547).

Bivalent or bispecific antibody fragments comprising a binding complex containing two polypeptide chains, one comprising two heavy chain variable domains (V_H) in series and the other comprising two light chain variable domains (V_L) in series are described in our pending European Patent Application No. 95307332.7.

Multivalent and/or multispecific antibody fragments are described in WO 94/09131 (Scotgen Limited). Specific binding proteins having two binding regions, contained at least in part on first and second polypeptide chains which chains additionally incorporate associating domains capable of binding to each other causing the polypeptide chains to combine are disclosed therein. It is disclosed that the first and second binding regions preferably are antibody antigen-binding domains, for example comprising V_H and V_L regions contained in a Fab fragment or in a single-chain Fv fragment, or may be derived from just one of the V_H or V_L regions of an antibody. The associating domains may suitably be derived from an antibody and may be inter alia antibody V_H and V_L regions. It is further disclosed that using a V_H/V_L domain combination to achieve association leads to the creation of a supplementary Fv domain such that the antibody produced may be trivalent. Schematic representations of the arrangements suggested in WO 94/09131 to produce trivalent fragments are shown in Figure 1A.

WO 93/11161 (Enzon Inc) describes multivalent antigen-binding proteins comprising two or more single-chain protein molecules, each single chain molecule comprising first and second polypeptides each comprising the binding portion of the variable region of an antibody heavy or light chain with the polypeptides being linked together via a peptide linker. Hypothetical trimers and tetramers are discussed, comprising three or four single-chain antigen binding proteins as appropriate. Schematic representations of the trivalent arrangements suggested are shown in Figure 1B.

WO 91/19739 (Celltech Limited) discloses multivalent antigen binding proteins comprising an Fv fragment bound to at least one further Fv fragment by a connecting structure which links the Fv fragments together but which maintains them spaced apart such that they can bind to adjacent antigenic determinants. Conveniently the connecting structure consists of a spacing polypeptide and a linkage unit such as a cross-linking maleimide linker or a molecule which allows for non-covalent binding. Particularly preferred connecting structures which are disclosed are based on antibody joining and hinge region sequences.

SUMMARY OF THE INVENTION

According to the present invention there is provided a multivalent antigen binding protein comprising:

a first polypeptide comprising in series, three or more variable domains of an antibody heavy chain; and

a second polypeptide comprising, in series, three or more variable domains of an antibody light chain,

said first and second polypeptides being linked by association of the respective heavy chain and light chain variable domains, each associated variable domain pair forming an antigen binding site.

As used herein, the term multivalent means more than one antigen binding site.

Preferably the first polypeptide comprises three variable domains of an antibody heavy chain and the second polypeptide comprises three variable domains of an antibody light chain, providing a trivalent protein.

It will be appreciated that the polypeptides may comprise heavy or light chains, variable domains, as appropriate, or functional equivalents thereof.

The respective heavy or light chain variable domains may suitably be linked without any intervening linker. According to a preferred embodiment, however, the variable domains contained in the individual polypeptides are linked by peptide linkers. Preferably the peptide linker is flexible, allowing the variable domains to flex in relation to each other such that they can bind to multiple antigenic determinants simultaneously. It will be appreciated that the binding of the linker to the individual heavy or light chain variable domains will be such that it does not affect the binding capacity of the binding site formed by the associated variable domain pair. Conveniently the peptide linker comprises from 16 to 19 amino acid residues. A preferred, peptide linker for heavy chain domains is $(Gly_4Ser)_3$AlaGlySerAla and for the light chain domains is $(Gly_4Ser)_3$Val.

It will be appreciated that if two or more of the associated variable domain pairs (V_H/V_L pairs) have the same antigen specificity, for example if they are derived

from the same parent antibody or fragment thereof or from different antibodies which bind the same epitope, then a binding protein which binds more than one molecule of the same type will be produced.

According to one embodiment, where the binding protein according to the invention comprises three antigen binding sites which are able to bind different epitopes from each other, a trivalent trispecific protein is produced.

In another embodiment, where the binding protein according to the invention comprises three associated variable domain pair binding sites, two of which sites bind the same epitopes, a trivalent, bispecific protein is provided. Where all three binding sites have the same antigen specificity, a trivalent, monospecific binding protein is provided.

The invention also provides nucleotide sequences coding for the polypeptides of the multivalent antigen binding protein according to the invention and cloning and expression vectors containing such nucleotide sequences.

The invention further provides host cells transformed with vectors containing such nucleotide sequences and methods of producing such polypeptides by expression of the nucleotide sequences in such hosts.

The invention further provides a process for preparing a multivalent antigen binding protein as set forth above comprising:

(i) transforming one or more hosts by incorporating genes encoding said first and second polypeptides;

(ii) expressing said genes in said host or hosts;

(iii) allowing said first and second polypeptides to combine to form the antigen binding protein.

Suitably the host or hosts may be selected from prokaryotic bacteria, such as Gram-negative bacteria, for example *E.Coli*, and Gram-positive bacteria, for example *B. subtilis* or lactic acid bacteria, lower eukaryotes such as yeasts, for example belonging to the genera *Saccharomyces Kluyveromyces* or *Trichoderma*, moulds such as those belonging to the genera *Aspergillus* and *Neurospora* and higher eukaroytes, such as plants, for example tobacco, and animal cells, examples of which are myeloma cells and CHO, COS cells and insect cells. A particularly preferred host for use in connection with the present invention is COS (monkey kidney) cells.

Techniques for synthesising genes, incorporating them into hosts and expressing genes in hosts are well known in the art and the skilled person would readily be able to put the invention into effect using common general knowledge. Proteins according to the invention may be recovered and purified using conventional techniques such as affinity chromatography, ion exchange chromatography or gel filtration chromatography.

The activity of the multivalent binding proteins according to the invention may conveniently be measured by standard techniques known in the art such as enzyme-linked immunosorbant assay (ELISA), radioimmune assay (RIA) or by using biosensors.

The multivalent antigen binding proteins of the present invention may suitably be used in diagnostics or therapy for example in targeting a tumour cell with natural killer cells and cytotoxic agent. Other uses for which the multivalent binding proteins according to the invention are useful include those uses for which

antibodies or fragments thereof are commonly used, including for immunoassays and in purification. According to a particular preferred embodiment, multi-enzyme complexes may be assembled, at a target, for example a cell surface. As an illustration, multivalent binding proteins according to the invention may be used to target cell killing enzymes such as an oxidase (for example glucose oxidase) and peroxidase (for example horseradish peroxidase) to a target species which is an antigenic component of dental plaque, such as *S. sanguis* or *S. mutans*. Complexes comprising enzyme, coenzyme and target antigen may also conveniently be assembled.

Accordingly, the invention also provides compositions comprising the multivalent antigen binding proteins according to the invention, conveniently in combination with a cosmetically or pharmaceutically acceptable carrier, diluent or excipient. Methods of treatment using the multivalent antigen binding proteins according to the invention are also provided.

For use in diagnosis or therapy, the multivalent antigen binding proteins according to the invention may conveniently be attached to an appropriate diagnostically or therapeutically effective agent or carrier by methods conventional in the art.

An advantage of using multivalents antigen binding proteins according to the invention over multivalent binding proteins prepared by existing techniques known in the art is that the "self-assembling" association of the respective heavy and light chain variable domains to form the multivalent binding sites avoids the need for chemical coupling steps or the introduction of linking residues to stabilise the multivalent constructs, thereby minimising the risk of eliciting an immune response to such molecules when the resulting multivalent binding

proteins are used in therapy.

A particular advantage of molecules according to the present invention is that they may conveniently be purified straight from the supernatant using conventional purification techniques. As they are self-assembling, there is no need to purify individual subunits prior to coupling as in existing techniques.

The present invention may be more fully understood with reference to the following description, when read together with the accompanying drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1A and 1B show schematic representations of published arrangements of heavy and light chain V-domain gene fragments that have been suggested to produce trispecific or trivalent antibody fragments:

A)	scFv1-VLa + scFv2-VHa (2 chains)	WO 94/09131
B)	Fab1-Vla + Fab2-VHa (4 chains)	WO 94/09131
C)	scFv1-VLa-CLa + scFv1-VHa-CHa (2 chains)	WO 94/09131
D)	Fab1-VLa-CLa + Fab2-VHa-CHa (4 chains)	WO 94/09131
E)	scFv1 + scFv2 + scFv3 (3 chains)	WO 93/11161
F)	VH1-VL2 + VH2-VL3 + VH3-VH1 (3 chains)	WO 93/11161

Figure 2A/B shows the nucleotide sequence of the EcoRI-HindIII insert of pGOSA.E2t containing DNA encoding pelB leader-VH4715-linker-VL3418 and DNA encoding pelB leader-VL3418-linker-VH4715-hydrophil2 tag (SEQ ID No. 1).

Figure 3

A) shows the nucleotide sequence of the HindIII-EcoRI insert of plasmid scFv.Lys with DNA encoding pelB leader-VHLys-linker-VLLys (SEQ ID No. 2).

B) shows the nucleotide sequence of the HindIII-EcoRI insert of plasmid scFv.4715.2t with DNA encoding pelB leader-VH4715.2t (SEQ ID No. 3).

Figure 4 shows the nucleotide sequence of the genomic leader sequence of the anti-NP antibody (Jones et al, Nature, 321, 522). Exon sequences are indicated with shaded boxes. NcoI and PstI restriction sites are in bold and underlined (SEQ ID No. 4).

Figure 5 gives a schematic representation of the eukaryotic expression vector pSV.51.

Figure 6 gives an overview of the pUC19 double head (A) and triple head (B) constructs. The position of the oligonucleotides and the restriction sites used for assembling double and triplehead pUC constructs are indicated.

Figure 7

A) shows the origin of the VH-C-linker and VL-C-linker fragments.

B) gives a schematic representation of the construction of the pUC.19-triple-head vectors.

Figure 8

A) gives a schematic representation of the construction of the Euka.VH and Euka.VL vectors.

B) gives a schematic representation of the construction of the pSV.VH expression vectors.

C) gives a schematic representation of the construction of the pSV.VL expression vectors.

Figure 9 shows the expression of the trispecific Golysan proteins on an SDS-PAGE gel containing total COS culture supernatant. Crude supernatants of COS cells transfected with pSV expression vectors were separated on SDS-PAGE gels. The proteins were transferred onto a nitrocellulose membrane and the VH3 and VL3-2t were detected using anti-VH and anti-hydrophil 2 tag specific monoclonal antibodies respectively. (A=anti-Hydro-II, B=anti-Hydro-II + anti-VH) Samples: M) Low Molecular Weight Markers, 1) pSV.K + pSV.V,2) pSV.K + pSV.W,3) pSV.M + pSV.V,4) pSV.M + pSV.W.

Figure 10 shows the results of three ELISA's. Lysozyme, Glucose oxidase and S.sanguis binding activity was determined in crude COS supernatants by measuring 1) Lysozyme-Glucose oxidase (=LYSOX), 2) Glucose oxidase-S.sanguis (=GOSA) and 3) Lysozyme-S.sanguis (= LYSAN) bispecific binding activities.

Figure 11 shows the results of three ELISA's. Lysozyme, Glucose oxidase and S.sanguis binding activity of purified Golysan.A (A) and Golysan.B (B) was determined by measuring 1) Lysozyme-Glucose oxidase (=LYSOX), 2) Glucose oxidase-S.sanguis (=GOSA) and 3) Lysozyme-S.sanguis (=LYSAN) bispecific binding activities.

Figure 12 shows the nucleotide sequence of the *Eco*RI-*Hind*III insert of pUR.4124 containing DNA (see SEQ ID NO: 23) encoding V_LLys-Linker-V_HLys.

Figure 13 shows the nucleotide sequence of the *Hind*III-*Eco*RI insert of plasmid Fv.3418 (see SEQ ID NO: 24) containing DNA encoding pelB leader-V_H3418 and pelB leader-V_L3418.

Figure 14 shows the nucleotide sequence of the *Hind*III-*Eco*RI insert of plasmid Fv.4715-myc (see SEQ ID NO: 25)

containing DNA encoding pelB leader-V_H4715 and pelB leader-V_L4715-Myc tag.

Figure 15 shows the nucleotide sequence of the *Hin*dIII-*Eco*RI insert of scFv.4715-myc containing DNA (see SEQ ID NO: 26) encoding pelB leader-V_H4715-Linker-V_L4715-Myc tag.

Figure 16a/b shows the nucleotide sequence of the *Hin*dIII-*Eco*RI insert of pGOSA.E (see SEQ ID NO: 27) containing DNA encoding pelB leader-V_H4715-Linker-V_L3418 and pelB leader-V_L3418-Linker-V_H4715.

Figure 16c gives an overview of the oligonucleotides and their positions in pGOSA.E that can be used to replace V-domain gene fragments.

Figure 17 shows the construction of plasmid pGOSA.A.

Figure 18 shows the construction of plasmid pGOSA.B.

Figure 19 shows the construction of plasmid pGOSA.C.

Figure 20 shows the construction of plasmid pGOSA.D.

Figure 21 shows the construction of plasmid pGOSA.E.

Figure 22 shows the source of fragment PCR.I *Bst*EII/*Sac*I.

Figure 23 shows the source of fragment PCR.IV *Xho*I/*Eco*RI.

Figure 24 shows the source of fragment PCR.V *Sal*I/*Eco*RI.

Figure 25 shows the source of fragment PCR.III *Nhe*I/*Sac*I.

Figure 26 shows the source of fragment PCR.II *Sfi*I/*Eco*RI.

Table 1 shows the nucleotide sequence of all

oligonucleotides used in the construction of the described double and triple head constructs.

Table 2 lists all pSV expression constructs described in this specification.

The following examples are provided by way of illustration only:

EXAMPLES

General Experimental

Strains, Plasmids and Media

All cloning steps were performed in E.Coli JM109 or E.Coli XL-1 Blue. Cultures were grown in 2xTY/Amp/Glucose medium (16g tryptone, 10g yeast extract, 5g NaCL per liter H_2O supplemented with 2% glucose and 100µg/ml ampicillin). Transformations were plated out on SOBAG plates (20g tryptone, 5g yeast extract, 15g agar, 0.5g NaCl per liter H_2O plus 10mM $MgCl_2$, 2% glucose, 100µg/ml ampicillin). The bicistronic E.coli vectors used are derivatives of pUC19. The COS expression vector pSV.51 (LMBP strain nr 1829) was obtained from the LMBP Culture collection (Laboratory of Molecular Biology University Gent). COS-1 cells (ECACC No: 88031701; African green monkey kidney cells) were obtained from the European Collection of Animal Cell Cultures (ECACC). All tissue culture reagents were from Gibco BRL (Life Technologies, Paisley, UK)

DNA Manipulations

Oligonucleotides and PCR

The oligonucleotide primers used in the PCR reactions were synthesized on an Applied Biosystems 381A DNA Synthesiser by the phosphoramidite method. The primary structures of the oligonucleotide primers used in the

construction of the trispecific pSV constructs (Table 2) are shown in Table 1. Reaction mixture used for amplification of DNA fragments were 10mM Tris-HCl, pH8.3, 2.5mM $MgCl_2$, 50mM KCl, 0.01% gelatin (w/v), 0.1% Triton X-100, 400mM of each dNTP, 5.0 units of Vent DNA polymerase (New England Biolabs), 100ng of template DNA, and 500ng of each primer (for 100µl reactions). Reaction conditions were: 94°C for 4 minutes, followed by 33 cycles of each 1 minute at 94°C, 1 minute at 55°C, and 1 minute 72°C.

Plasmid DNA\Vector\Insert preparation and ligation\transformation.

Plasmid DNA was prepared using the 'Qiagen P-100 and P-500 Midi/Maxi-DNA Preparation' system. Vectors and inserts were prepared by digestion of 10µg (for vector preparation) or 20µg (for insert preparation) with the specified restriction endonucleases under appropriate conditions (buffers and temperatures as specified by suppliers). Klenow fill-in reactions and dephosphorylation with Calf Intestine Phosphorylase were performed according to the manufacturers instructions. Vector DNA's and inserts were separated through agarose gel electrophoresis and purified with DEAE-membranes NA45 (Schleicher & Schnell) as described by Maniatis et al. (Molecular cloning: a Laboratory manual, Cold Spring Harbour, N.Y. (1982)) Ligations were performed in 20µl volumes containing 30mM Tris-HCl pH7.8, 10mM $MgCl_2$, 10mM DTT, 1mM ATP, 300-400ng vector DNA, 100-200ng insert DNA and 1 Weiss unit T_4 DNA ligase. After ligation for 2-4 h at room temperature, $CaCl_2$ competent E. coli JM109 or XL-1 Blue (Maniatis et al) were transformed using 7.5µl ligation reaction. The transformation mixtures were plated onto SOBAG plates and grown overnight at 37°C. Correct clones were identified by restriction analysis and verified by automated dideoxy sequencing (Applied Biosystems).

Restriction digestion of PCR products

Following amplification each reaction was checked for the presence of a band of the appropriate size by agarose gel electrophoresis. One or two 100µl PCR reaction mixtures of each of the PCR reactions, together containing approximately 2-4µg DNA product were subjected to phenol-chloroform extraction, chloroform extraction and ethanol precipitation. The DNA pellets were washed twice with 70% ethanol and allowed to dry. Next, the PCR products were digested overnight (18 h) in 200µL 1xBuffer with excess of the appropriate restriction enzyme.

Transformation of COS Cells

Cos-1 cells were maintained in DMEM culture medium with glutamine (2mM), Penicillin (100U/mL), streptomycin (100µg/mL) containing 10% F.C.S. For transient transfection assays 1-3 x10^5 COS-1 cells were seeded in 3 cm-diameter tissue culture dishes (2mL). The cells were incubated at 37°C in a CO_2 incubator until cells were 50-80% confluent (overnight). For each transfection the following mixes were prepared: A) 1µg of each of the specified DNA's in 100µL Opti-MEM-I Reduced Serum Medium, B) 1µL LipofectAmine in 100µL Opti-MEM-I Reduced Serum Medium. Mixes A and B were combined (gently). After allowing the DNA-liposome complexes to form for 30-45 minutes at room temperature, 0.8mL Opti-MEM-I Reduced Serum Medium was added to each lipid DNA complex containing tube. The COS-1 cells were washed once with 2mL of Opti-MEM-I Reduced Serum Medium and overlayed with the diluted complex solution. The COS-1 cells were incubated for 5 hr at 37°C. Following incubation, 2mL growth medium was added. 20 hours following transfection the medium was replaced with 2mL fresh growth medium containing 0.1mM Na-butyrate. After 48 hours incubation at 37°C the supernatant was harvested and assayed for the presence of antibody fragments.

ELISA

A) GOSA: Glucose Oxidase and S.sanguis binding activity

96 well ELISA plates (Greiner HC plates) were activated overnight at 37°C with 200µl/well of a 1/10 dilution of an overnight culture of Streptococcus sanguis cells in 0.05M sodium carbonate buffer pH9.5 was used to sensitise each well. Following one wash with PBST, the antigen sensitised plates were pre-blocked for 1 hour at 37°C with 200µl/well blocking buffer (1% BSA, 0.15% Tween in PBS). 50µl COS culture supernatants (neat or diluted with PBS) plus 50µl blocking buffer containing glucose oxidase (50µg/ml) was added to the Streptococcus Sanguis sensitised plate and incubated for 2 hours at 37°C. Following 4 washes with PBS-T, bound glucose oxidase was detected by adding 100µl substrate to each well (70mM Na-citrate, 320mM Na-phosphate, 27mg/ml glucose, 0.5µg/ml HRP, 100µg/ml TMB). The colour reaction was stopped after 1 hour by the addition of 35µl 2M HCl and the A450 was measured.

B) LYSOX: Lysozyme and Glucose Oxidase binding activity

96 well ELISA plates (Greiner HC plates) were activated overnight at 37°C with lysozyme (50µg/mL in 0.05M sodium carbonate buffer pH9.5; 200µl/well). Following one wash with PBST, the antigen sensitised plates were pre-blocked for 1 hour at 37°C with 200µl/well blocking buffer (1% BSA, 0.15% Tween in PBS). 50µl COS culture supernatants (neat or diluted with PBS) plus 50µl blocking buffer containing glucose oxidase (50µg/ml) was added to the Streptococcus Sanguis sensitised plate and incubated for 2 hours at 37°C. Following 4 washes with PBS-T, bound glucose oxidase was detected by adding 100µl substrate to each well (70mM Na-citrate, 320mM Na-phosphate, 27mg/ml glucose, 0.5µg/ml HRP, 100µg/ml TMB). The colour reaction was stopped after 1 hour by the addition of 35µl 2M HCl and the A450 was measured.

C) LYSAN: S.sanguis and Lysozyme binding activity

96 well ELISA plates (Greiner HC plates) were activated overnight at 37°C with 200µl/well of a 1/10 dilution of an overnight culture of Streptococcus sanguis cells in 0.05M sodium carbonate buffer pH9.5 was used to sensitise each well. Following one wash with PBST, the antigen sensitised plates were pre-blocked for 1 hour at 37°C with 200µl/well blocking buffer (1% BSA, 0.15% Tween in PBS). 50µl COS culture supernatants (neat or diluted with PBS) plus 50µl blocking buffer was added to the Streptococcus Sanguis sensitised plate and incubated for 2 hours at 37°C. Following 4 washes with PBS-T, 50µL blocking buffer containing Alkaline-Phosphatase conjugated Lysozyme (100µ/mL). Unbound Lysozyme was removed by 4 washes with PBS-T. Bound Lysozyme was detected by adding 100µL substrate solution to each well (1mg/ml pNPP in 1M diethanolamine, 1mM $MgCl_2$). After 1 hour the A405 was measured.

EXAMPLE 1: Construction of the pSV.Golysan expression vectors

The construction of the pSV COS expression vectors consisted of three stages:

1A): Assembly of 2 heavy chain variable domains and 2 light chain variable domains in a pUC based E.Coli expression vector thus constructing the VH_A-VH_B and VL_A-VL_B modules respectively.

1B): Assembly of 3 heavy chain variable domains and 3 light chain variable domains in a pUC based E.Coli expression vector thus constructing the VH_A-VH_B-VH_C and VL_A-VL_B-VL_C modules respectively.

2) Linking the VH_A-VH_B, VH_A-VH_B-VH_C and VL_A-VL_B, VL_A-VL_B-VL_C to the genomic anti-NP leader sequence in the

intermediate "EUKA" vectors to ensure efficient secretion by COS cells.

3) Inserting the leader-VH_A-VH_B, leader-VH_A-VH_B-VH_C and leader-VL_A-VL_B, leader-VL_A-VL_B-VL_C as XbaI/XbaI fragments downstream of the SV40 promoter in the COS expression vector pSV.51.

ad.1) E.coli expression vectors.

The E.coli expression vectors are derivatives of pUC.19 containing a HindIII-EcoRI fragment that in the case of the scFv.lys-myc contains a pelB signal sequence fused to the 5' end of the heavy chain V-domain that is directly linked to the corresponding light chain V-domain of the antibody through a connecting sequence that codes for a flexible peptide $(Gly_4Ser)_3$ thus generating a single-chain molecule. In the 'double head' expression vector both the heavy chain and the light chain V-domains of the antibody are preceded by a ribosome binding site and a pelB signal sequence in an artificial dicistronic operon under the control of a single inducible promoter. Expression of these constructs is driven by the inducible lacZ promoter. The nucleotide sequence of the HindIII-EcoRI inserts of the scFv.lys-myc, scFv.4715.2t and pGOSA.E2t constructs used for the generation of the trispecific antibody fragments are listed in Figures 3 and 2 respectively.

ad.1A) Assembly of bi-specific fragments or double heads.

The construct pGOSA.E2t (Figures 2 and 6A) is derived from the E.coli expression construct pGOSA.E. The construction of pGOSA.E has been described in detail in preparation 1 below.

In contrast with pGOSA.E, pGOSA.E2t contains a peptide tag at the C-terminus of the Variable light chain. Using oligonucleotides DBL3 and DBL.4 the VL4715 gene fragment

was amplified using scFv.4715.2t as a template. The SalI/BamHI VH4715.2t PCR fragment and the Hydrophil-2 tag containing BamHI/EcoRI fragment from scFv.4715.2t (Figure 3B) were used to replace the SalI/EcoRI VH4715 fragment in pGOSA.E thus producing pGOSA.E2t.

The vector pGOSA.E2t and the oligonucleotides in Table 1 have been designed to enable most specificities to be cloned into the pGOSA.E2t construct (Figure 6A). The upstream V_H domain can be replaced by any PstI-BstEII V_H gene fragment obtained with oligonucleotides PCR.51 and PCR.89. The oligonucleotides DBL.1 and DBL.2 were designed to introduce SfiI and NheI restriction sites in the V_H gene fragments thus allowing cloning of those V_H gene fragments into the SfiI-NheI sites as the downstream V_H domain. Using this approach the following VH_A-VH_B combinations were constructed: VH4715-VH3418, VH4715-VHlys, VH3418-VHlys, VHlys-VH3418.

All V_L gene fragments obtained with oligonucleotides PCR.116 and PCR.90 can be cloned into the position of the 3418 V_L gene fragment as a SacI-XhoI fragment. A complication here however is the presence of an internal SacI site in the 3418 V_H gene fragment. Oligonucleotides DBL.3 and DBL.4 are designed to allow cloning of V_L gene fragments into the position of the 4715 V_L gene fragment as a SalI-BamHI fragment. A complication here however is the presence of an internal BamHI site in the hydrophil-2-tag gene fragment (2t). Using this approach the following VL_A-VL_B combinations were constructed: VL3418-VL4715.2t, VLlys-VL4715.2t and VLlys-VL3418.2t.

ad.1B) Assembly of tri-specific fragments or triple heads.

Amplification of the VH-linker fragments using either scFv (VH-linker-VL) or bi-specific constructs (VH-linker-VH) as template with the primer combination DBL.1/DBL.5

(Figure 7A) yields one of the building blocks for the construction of the VH_A-VH_B-VH_C modules. The VH-linker DBL.1/DBL.5 PCR fragment is digested with SfiI and inserted into the SfiI site that is present between the linker sequence and the downstream VH domain in all bi-specific constructs (Figure 7B) thus producing a VH_A-VH_B-VH_C module. Using this approach the following VH_A-VH_B-VH_C combinations were constructed for this filing: VH4715-VHlys-VH3418 and VHlys-VH4715-VH3418.

Using a bi-specific construct (VL-linker-VL_L) as the template in an amplification reaction with the primer combination DBL.3/DBL.6 (Figure 7A) yields the VL-linker building block for the construction of the VL_A-VL_B-VL_C modules. The VL-linker DBL.3/DBL.6 PCR fragment is digested with SalI and inserted into the SalI site that is present between the linker sequence and the downstream VL domain in all bi-specific constructs (Figure 7B) thus producing a VL_A-VL_B-VL_C module. Using this approach the following VL_A-VL_B-VL_C combinations were constructed: VLlys-VL4715-VL3418.2t and VL3418-VLlys-VL4715.2t.

A schematic representation of the final tri-specific constructs is shown in Figure 6B.

ad.2) Linking the variable region domains to the leader sequence.

The HindIII/EcoRI polylinker of pUC19 was replaced with a synthetic EcoRI/HindIII 'Euka' polylinker. This was achieved by annealing and inserting the synthetic oligonucleotides Euka.1 and Euka.2 (Table 1) into EcoRI/HindIII digested pUC19 vector. The resulting Euka.pUC vector contains all restriction sites needed for the subcloning of the leader sequence and the VH and VL domains. The NcoI/PstI genomic anti-NP leader sequence fragment was cloned into the NcoI/PstI digested Euka.pUC vector yielding the Euka.VH construct (Figure 8A).

Oligonucleotides ML.1 and ML.2 (Table 1) were used in an amplification reaction to introduce a SacI site at the 3' end of the leader sequence that allows the construction of leader-VL fusions. The NcoI/SacI leader sequence PCR fragment was inserted into NcoI/SacI digested Euka.pUC vector yielding the Euka.VL construct (Figure 8A).

The VH_A-VH_B and VH_A-VH_B-VH_C modules were excised from the pUC expression vectors as PstI/NheI fragments and inserted into PstI/NheI digested Euka.VH vector (Figure 8B). Using this approach the following leader-VH_A-VH_B and leader-VH_A-VH_B-VH_C combinations were constructed for this filing: Euka.B: leader-VH4715-VH3418, Euka.D: leader-VH4715-VHlys, Euka.G: leader-VH3418-VHlys, Euka.K: leader-VH4715-VHlys-VH3418 and Euka.M: leader-VHlys-VH4715-VH3418.

The VL_A-VL_B and VL_A-VL_B-VL_C modules were excised from the pUC expression vectors as EcoRI-Klenow/SacI fragments and inserted into NotI-Klenow/SacI treated Euka.VL vector (Figure 8C). Using this approach the following leader-VL_A-VL_B and leader-VL_A-VL_B-VL_C combinations were constructed: Euka.N: leader-VL3418-VL4715.2t, Euka.P: leader-VLlys-VL4715.2t Euka.S: leader-VLlys-VL3418.2t, Euka.V: leader-VLlys-VL4715-VL3418.2t and Euka.W: leader-VL3418-VLlys-VL4715.2t.

ad.3) Subcloning of leader-variable domain fusions into the pSV.51 expression vector

All leader-VH_A-VH_B, leader-VH_A-VH_B-VH_C, leader-VL_A-VL_B and leader-VL_A-VL_B-VL_C combinations were excised from the 'Euka' vectors as XbaI/XbaI fragments and subcloned downstream of the SV40 promoter in pSV.51 (Figure 5) by insertion into the XbaI site (Figure 8B and 8C). After confirmation of the correct orientation of the inserts the pSV expression vectors were used to transfect COS-1 cells (see Example 2). The pSV expression vectors used

are listed in Table 2.

Example 2: Bifunctional binding activity of Golysan triple heads

This example describes the production of three types of bispecific binding activity by COS-1 cells transfected with expression plasmids encoding the corresponding VH_A-VH_B-VH_C and VL_A-VL_B-VL_C genes fragments.

1. Production of antibody fragments by COS-1 cells

Supernatants of COS-1 cells transfected with combinations of pSV-VH_A-VH_B-VH_C and pSV-VL_A-VL_B-VL_C expression plasmids were separated on 10% SDS-PAGE and transferred onto nitrocellulose. The resulting Western blots were screened with a monoclonal antibody recognising a peptide sequence in framework 4 of the VH domains (region encoded by PCR.89: conserved in all used VH domains, {in-house reagent}) and/or a monoclonal specific for the hydrophil-2 tag. As shown in Figure 9 all supernatants contained products with the expected molecular weight of the VH_A-VH_B-VH_C and VL_A-VL_B-VL_C fragments, indicating that the COS cells were successfully tranfected and were secreting the produced antibody fragments into the culture medium at detectable levels.

2. Bifunctional binding activity

Supernatants of COS-1 cells transfected with single pSV expression plasmids and combinations of pSV expression plasmids were tested for the production of bifunctional binding activity using ELISA format:

* Supernatants of COS-1 cells transfected with the bispecific positive controls 'LYSAN' (pSV.D + pSV.P), 'LYSOX' (pSV.G + pSV.S) and 'GOSA' (pSV.B + pSV.N) only produced LYSAN, LYSOX and GOSA bispecific activity respectively (Figure 10). No significant cross

reactivity was detected.

* Supernatants of COS-1 cells transfected with only one expression vector encoding either one of the VH_A-VH_B-VH_C fragments (pSV.K and pSV.M) or one of the VL_A-VL_B-VL_C fragments (pSV.V and pSV.W) did not exhibit any bispecific binding activity, indicating that no background binding or a specific binding activity is produced.

* All tested supernatants of COS-1 cells transfected with an expression vector encoding one of the VH_A-VH_B-VH_C fragments (pSV.K and pSV.M) and an expression vector encoding one of the VL_A-VL_B-VL_C fragments (pSV.V and pSV.W) showed significant levels of all three bifunctional binding activities LYSOX, GOSA and LYSAN.

These results show that COS cells transfected with expression vectors encoding VH_A-VH_B-VH_C and expression vectors encoding VL_A-VL_B-VL_C fragments produce and secrete molecules that contain three binding activities. In this example those three activities are: Glucose Oxidase binding, S.sanguis binding and Lysozyme binding. Furthermore, the results illustrated in Figure 10 clearly show that at least two of these binding activity are present in one self assembling molecular complex. In this example those combinations are: GOSA (Glucose Oxidase + S.sanguis), LYSOX (Lysozyme + Glucose Oxidase) and LYSAN (Lysozyme + S.sanguis).

Example 3: Trifunctional binding activity of Golysan triple heads

This example describes experiments that show that the three types of bispecific binding activity that are produced by COS-1 cells transfected with expression plasmids encoding the corresponding VH_A-VH_B-VH_C and VL_A-

VL_B-VL_C genes fragments are present in one self assembling molecular complex.

Golysan.A (VHlys-VH4715-VH3418 + VLlys-VL4715-VL3418.2t) and Golysan.B (VHlys-VH4715-VH3418 + VL3418-VLlys-VL4715.2t) was purified by affinity chromatography. 100ml supernatant of COS-1 cells transfected with expression plasmids pSV.M/pSV.V (Golysan.A) or pSV.M/pSV.W (Golysan.B) were loaded onto a Lysozyme-Sepharose column (CNBr-Sepharose, Pharmacia; column was prepared according to the manufacturer's instructions). After extensive washes with PBS the bound Golysan antibody fragments were eluted in 0.1M glycine buffer at pH=2.2. The fractions were neutralised with Tris and tested for the presence of trispecific binding activity.

As shown in Figure 11 no bispecific binding activity was detect in the column fall-through. All three bispecific binding activities (GOSA, LYSOX and LYSAN) were extracted from the COS-1 supernatant by passing over the Lysozyme affinity matrix. After acid elution all three bispecific binding activities (GOSA, LYSOX and LYSAN) were recovered from the column. Since both Golysan.A and B were affinity purified based on the ability to bind to Lysozyme, the finding that these molecules also bind S.sanguis and Glucose Oxidase shows that all three binding activities are present in one self assembling molecular complex.

Preparation 1.
Construction of the pGOSA.E double head expression vector

In the pGOSA expression vectors, the DNA fragments encoding both the V_H and V_L of the antibody are preceded by a ribosome binding site and a DNA sequence encoding the pelB signal sequence in an artificial dicistronic operon under the control of a single inducible promoter.

Expression of these constructs is driven by the inducible lacZ promoter. The nucleotide sequence of the *Hin*dIII-*Eco*RI inserts of the plasmids pUR.4124 (SEQ ID NO. 23), Fv.3418 (SEQ ID NO. 24), Fv.4715-myc (SEQ ID NO. 25) and scFv.4715-myc (SEQ ID NO. 26) constructs used for the generation of the bispecific antibody fragments are given in Figures 12-15, respectively. Moreover, a culture of *E. coli* cells harbouring plasmid scFv.4715-myc and a culture of *E. coli* cells harbouring plasmid Fv.3418 were deposited under the Budapest Treaty at the National Collection of Type Cultures (Central Public Health Laboratory) in London (United Kingdom) with deposition numbers NCTC 12916 and NCTC 12915, respectively.

In agreement with Rule 28 (4) EPC, or a similar arrangement for a State not being a Contracting State of the EPC, it is hereby requested that a sample of such deposit, when requested, will be submitted to an expert only.

The construction of pGOSA.E (see Figure 16 for the *Hin*dIII-*Eco*RI insert of pUC19) involved several cloning steps. The appropriate restriction sites in the various domains were introduced by PCR directed mutagenesis using the oligonucleotides listed in Table 1 below.

The construction of pGOSA.E involved several cloning steps that produced 4 intermediate constructs pGOSA.A to pGOSA.D (see FigureS 17-21). The final expression vector pGOSA.E and the oligonucleotides in Table 1 have been designed to enable most specificities to be cloned into the final pGOSA.E construct (Figure 16c). The upstream V_H domain can be replaced by any *Pst*I-*Bst*EII V_H gene fragment obtained with oligonucleotides PCR.51 and PCR.89 (see Table 1). The oligonucleotides DBL.1 and DBL.2 (see Table 1) were designed to introduce *Sfi*I and *Nhe*I restriction sites in the V_H gene fragments thus allowing

cloning of those V_H gene fragments into the *Sfi*I-*Nhe*I sites as the downstream V_H domain. All V_L gene fragments obtained with oligonucleotides PCR.116 and PCR.90 (see Table 1) can be cloned into the position of the V_L.3418 gene fragment as a *Sac*I-*Xho*I fragment. A complication here however is the presence of an internal *Sac*I site in the V_H.3418 gene fragment. Oligonucleotides DBL.3 and DBL.9 (see Table 1) are designed to allow cloning of V_L gene fragments into the position of the V_L.4715 gene fragment as a *Sal*I-*Not*I fragment.

pGOSA.A

This plasmid is derived from both the Fv.4715-myc construct (SEQ ID NO. 25) and the scFv.4715-myc construct (SEQ IN NO. 26). An *Sfi*I restriction site was introduced between the DNA sequence encoding the $(Gly_4Ser)_3$ linker and the gene fragment encoding the V_L of the scFv.4715-myc construct (see Figure 17). This was achieved by replacing the *Bst*EII-*Sac*I fragment of the latter construct by the fragment PCR-I *Bst*EII/*Sac*I (Figure 22) that contains an *Sfi*I site between the DNA encoding the $(Gly_4Ser)_3$ linker and the V_L.4715 gene fragment. The introduction of the *Sfi*I site also introduced 4 additional amino acids (AlaGlySerAla) between the $(Gly_4Ser)_3$ linker and V_L.4715 resulting in a $(Gly_4Ser)_3$AlaGlySerAla linker (linkerA). The oligonucleotides used to produce PCR-I (DBL.5 and DBL.7, see Table 1) were designed to match the sequence of the framework-3 region of V_H.4715 and to prime at the junction of the DNA encoding the $(Gly_4Ser)_3$ linker and the V_L.4715 gene fragment, respectively. Thus pGOSA.A can be indicated as:

***pel*B-V_H4715-linkerA-(*Sfi*I)-V_L4715-myc.**

pGOSA.B

This plasmid is derived from plasmid Fv.3418 (see Figure 18). The *Xho*I-*Eco*RI fragment of plasmid Fv.3418

comprising the 3' end of DNA encoding framework-4 of the V_L including the stop codon was removed and replaced by the fragment PCR-IV *Xho*I/*Eco*RI (Figure 23). The oligonucleotides used to produce PCR-IV (DBL.8 and DBL.6, see Table 1) were designed to match the sequence at the junction of the V_L and the $(Gly_4Ser)_3$ linker perfectly (DBL.8), and to be able to prime at the junction of the $(Gly_4Ser)_3$ linker and the V_H in pUR.4124 (DBL.6). DBL.6 removed the *Pst*I site in the V_H (silent mutation) and introduced a *Sal*I restriction site at the junction of the $(Gly_4Ser)_3$ linker and the V_H, thereby replacing the last Ser of the linker by a Val residue resulting in a $(Gly_4Ser)_2Gly_4Val$ linker (linkerV). Thus pGOSA.B can be indicated as:

***pel*B-V_H3418 + *pel*B-V_L3418-linkerV-(*Sal*I-*Eco*RI).**

pGOSA.C

This plasmid contains DNA encoding V_H.4715 linked by the $(Gly_4Ser)_3$AlaGlySerAla linker to V_H.3418 (see Figure 19), thus:

***pel*B-V_H4715-linkerA-V_H3418.**

This construct was obtained by replacing the *Sfi*I-*Eco*RI fragment from pGOSA.A encoding V_L.4715 by the fragment PCR-II *Sfi*I/*Eco*RI containing the V_H.3418 gene. The oligonucleotides used to produce PCR-II (DBL.1 and DBL.2, see Table 1) hybridize in the framework-1 and framework-4 region of the gene encoding V_H.3418, respectively. DBL.1 was designed to remove the *Pst*I restriction site (silent mutation) and to introduce an *Sfi*I restriction site upstream of the V_H gene. DBL.2 destroys the *Bst*EII restriction site in the framework-4 region and introduces an *Nhe*I restriction site downstream of the stopcodon.

pGOSA.D

This plasmid contains a dicistronic operon comprising the V_H.3418 gene and DNA encoding V_L.3418 linked by the

$(Gly_4Ser)_2Gly_4Val$ linker to V_L.4715 (see Figure 20), thus:
pelB-V$_H$3418 + pelB-V$_L$3418-linkerV-V$_L$4715.

This construct was obtained by digesting plasmid pGOSA.B with *Sal*I-*Eco*RI and inserting the fragment PCR-V *Sal*I/*Eco*RI (Figure 24) containing the V_L.4715 gene. The oligonucleotides used to obtain PCR-V (DBL.3 and DBL.9, see Table 1) were designed to match the nucleotide sequence of the framework-1 and framework-4 regions of the V_L.4715 gene, respectively. DBL.3 removed the *Sac*I site from the framework-1 region (silent mutation) and introduced a *Sal*I restriction site upstream of the V_L.4715 gene. DBL.9 destroyed the *Xho*I restriction site in the framework-4 region of the V_L.4715 gene (silent mutation) and introduced a *Not*I and an *Eco*RI restriction site downstream of the stop codon.

pGOSA.E

This plasmid contains a dicistronic operon comprising DNA encoding V_H.4715 linked by the $(Gly_4Ser)_3AlaGlySerAla$ linker to V_H.3418 plus DNA encoding V_L.3418 linked by the $(Gly_4Ser)_2Gly_4Val$ linker to V_L.4715 (see Figure 21), thus:
pelB-V$_H$4715-linkerA-V$_H$3418 + pelB-V$_L$3418-linkerV-V$_L$4715.

Both translational units are preceded by a ribosome binding site and DNA encoding a pelB leader sequence. This plasmid was obtained by a three-point ligation by mixing the vector resulting from pGOSA.D after removal of the V_H3418-encoding *Pst*I-*Sac*I insert with the *Pst*I-*Nhe*I pGOSA.C insert containing V_H.4715 linked to V_H.3418 and the PCR-III *Nhe*I/*Sac*I fragment (see Figure 25). The remaining *Pst*I-*Sac*I pGOSA.D vector contains the 5' end of the framework-1 region of V_H.3418 upto the *Pst*I restriction site and V_L.3418 linked by the $(Gly_4Ser)_2Gly_4Val$ linker to V_L.4715 starting from the *Sac*I restriction site in V_L.3418. The *Pst*I-*Nhe*I pGOSA.C insert contains V_H.4715 linked by the $(Gly_4Ser)_3AlaGlySerAla$

linker to V_H.3418, starting from the *Pst*I restriction site in the framework-1 region in V_H.4715. The *Nhe*I-*Sac*I PCR-III fragment provides the ribosome binding site and DNA encoding the *pel*B leader sequence for the V_L.3418-$(Gly_4Ser)_2Gly_4Val$-V_L.4715 construct. The oligonucleotides DBL.10 and PCR.116 (see Table 1) used to generate PCR-III were designed to match the sequence upstream of the ribosome binding site of V_L.4715 in Fv.4715 and to introduce an *Nhe*I restriction site (DBL.10), and to match the framework-4 region of V_L.3418 (PCR.116).

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:
(A) NAME: UNILEVER PLC
(B) STREET: Blackfriars
(C) CITY: London
(E) COUNTRY: United Kingdom
(F) POSTAL CODE (ZIP): EC4P 4BQ
(G) TELEPHONE: (01234) 222644
(H) TELEFAX: (01234) 222633
(I) TELEX: 82229 UNILAB G

(ii) TITLE OF INVENTION: Multivalent and multispecific antigen-binding protein

(iii) NUMBER OF SEQUENCES: 27

(iv) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE:
PatentIn Release #1.0, Version #1.25 (EPO)(SEC ID NO. 1 to 18)
PatentIn Release #1.0, Version #1.30 (EPO) (SEC ID NO. 19 to 27)

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1745 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

AAGCTTGCAT GGAAATTCTA TTTCAAGGAG ACAGTCATAA TGAAATACCT ATTGCCTACG 60

GCAGCCGCTG GATTGTTATT ACTCGCTGCC CAACCAGCGA TGGCCCAGGT GCAGCTGCAG 120

GAGTCAGGGG GAGACTTAGT GAAGCCTGGA GGGTCCCTGA CACTCTCCTG TGCAACCTCT 180

GGATTCACTT TCAGTAGTTA TGCCTTTTCT TGGGTCCGCC AGACCTCAGA CAAGAGTCTG 240

GAGTGGGTCG CAACCATCAG TAGTACTGAT ACTTATACCT ATTATTCAGA CAATGTGAAG 300

GGGCGCTTCA CCATCTCCAG AGACAATGGC AAGAACACCC TGTACCTGCA AATGAGCAGT 360

CTGAAGTCTG AGGACACAGC CGTGTATTAC TGTGCAAGAC ATGGGTACTA TGGTAAAGGC 420

TATTTTGACT ACTGGGGCCA AGGGACCACG GTCACCGTCT CCTCAGGTGG AGGCGGTTCA 480

GGCGGAGGTG GCTCTGGCGG TGGCGGATCG GCCGGTTCGG CCCAGGTCCA GCTGCAACAG 540

TCAGGACCTG AGCTGGTAAA GCCTGGGGCT TCAGTGAAGA TGTCCTGCAA GGCTTCTGGA 600

TACACATTCA CTAGCTATGT TATGCACTGG GTGAAACAGA AGCCTGGGCA GGGCCTTGAG 660

TGGATTGGAT ATATTTATCC TTACAATGAT GGTACTAAGT ACAATGAGAA GTTCAAAGGC 720

AAGGCCACAC TGACTTCAGA CAAATCCTCC AGCACAGCCT ACATGGAGCT CAGCAGCCTG 780

ACCTCTGAGG ACTCTGCGGT CTATTACTGT TCAAGACGCT TTGACTACTG GGGCCAAGGG 840

ACCACCGTCA CCGTCTCCTC ATAATAAGCT AGCGGAGCTG CATGCAAATT CTATTTCAAG 900

GAGACAGTCA TAATGAAATA CCTATTGCCT ACGGCAGCCG CTGGATTGTT ATTACTCGCT 960

GCCCAACCAG CGATGGCCGA CATCGAGCTC ACCCAGTCTC CATCTTCCAT GTATGCATCT 1020

CTAGGAGAGA GAATCACTAT CACTTGCAAG GCGAGTCAGG ACATTAATAC CTATTTAACC 1080

TGGTTCCAGC AGAAACCAGG GAAATCTCCC AAGACCCTGA TCTATCGTGC AAACAGATTG 1140

CTAGATGGGG TCCCATCAAG GTTCAGTGGC AGTGGATCTG GGCAAGATTA TTCTCTCACC 1200

ATCAGCAGCC TGGACTATGA AGATATGGGA ATTTATTATT GTCTACAATA TGATGAGTTG 1260

TACACGTTCG GAGGGGGGAC CAAGCTCGAG ATCAAACGGG GTGGAGGCGG TTCAGGCGGA 1320

GGTGGCTCTG GCGGTGGCGG AGTCGACATC GAACTCACTC AGTCTCCATT CTCCCTGACT 1380

GTGACAGCAG GAGAGAAGGT CACTATGAAT TGCAAGTCCG GTCAGAGTCT GTTAAACAGT 1440

GTAAATCAGA GGAACTACTT GACCTGGTAC CAGCAGAAGC CAGGGCAGCC TCCTAAACTG 1500

TTGATCTACT GGGCATCCAC TAGGGAATCT GGAGTCCCTG ATCGCTTCAC AGCCAGTGGA 1560

TCTGGAACAG ATTTCACTCT CACCATCAGC AGTGTGCAGG CTGAAGACCT GGCAGTTTAT 1620

TACTGTCAGA ATGATTATAC TTATCCGTTC ACGTTCGGAG GGGGGACCAA GCTCGAAATC 1680

AAACGGGGAT CCGGTAGCGG GAACTCCGGT AAGGGGTACC TGAAGTAATA AGCGGCCGCG 1740

AATTC 1745

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 894 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

AAGCTTGCAT GCAAATTCTA TTTCAAGGAG ACAGTCATAA TGAAATACCT ATTGCCTACG 60

GCAGCCGCTG GATTGTTATT ACTCGCTGCC CAACCAGCGA TGGCCCAGGT GCAGCTGCAG 120

GAGTCAGGAC CTGGCCTGGT GGCGCCCTCA CAGAGCCTGT CCATCACATG CACCGTCTCA 180

GGGTTCTCAT TAACCGGCTA TGGTGTAAAC TGGGTTCGCC AGCCTCCAGG AAAGGGTCTG 240

GAGTGGCTGG GAATGATTTG GGGTGATGGA AACACAGACT ATAATTCAGC TCTCAAATCC 300

AGACTGAGCA TCAGCAAGGA CAACTCCAAG AGCCAAGTTT TCTTAAAAAT GAACAGTCTG 360

CACACTGATG ACACAGCCAG GTACTACTGT GCCAGAGAGA GAGATTATAG GCTTGACTAC 420

TGGGGCGAAG GCACCACGGT CACCGTCTCC TCAGGTGGAG GCGGTTCAGG CGGAGGTGGC 480

TCTGGCGGTG GCGGATCGGA CATCGAGCTC ACCCAGTCTC CAGCCTCCCT TTCTGCGTCT 540

GTGGGAGAAA CTGTCACCAT CACATGTCGA GCAAGTGGGA ATATTCACAA TTATTTAGCA 600

TGGTATCAGC AGAAACAGGG AAAATCTCCT CAGCTCCTGG TCTATTATAC AACAACCTTA 660

GCAGATGGTG TGCCATCAAG GTTCAGTGGC AGTGGATCAG GAACACAATA TTCTCTCAAG 720

ATCAACAGCC TGCAACCTGA AGATTTTGGG AGTTATTACT GTCAACATTT TTGGAGTACT 780

CCTCGGACGT TCGGTGGAGG CACCAAGCTC GAGATCAAAC GGGAACAAAA ACTCATCTCA 840

GAAGAGGATC TGAATTAATA AGATCAAACG GTAATAAGGA TCCAGCTCGA ATTC 894

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 930 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

AAGCTTGCAT GCAAATTCTA TTTCAAGGAG ACAGTCATAA TGAAATACCT ATTGCCTACG 60

GCAGCCGCTG GATTGTTATT ACTCGCTGCC CAACCGGCCA TGGCCCAGGT GCAGCTGCAG 120

GAGTCAGGGG GAGACTTAGT GAAGCCTGGA GGGTCCCTGA CACTCTCCTG TGCAACCTCT 180

GGATTCACTT TCAGTAGTTA TGCCTTTTCT TGGGTCCGCC AGACCTCAGA CAAGAGTCTG 240

GAGTGGGTCG CAACCATCAG TAGTACTGAT ACTTATACCT ATTATTCAGA CAATGTGAAG 300

GGGCGCTTCA CCATCTCCAG AGACAATGGC AAGAACACCC TGTACCTGCA AATGAGCAGT 360

CTGAAGTCTG AGGACACAGC CGTGTATTAC TGTGCAAGAC ATGGGTACTA TGGTAAAGGC 420

TATTTTGACT ACTGGGGCCA AGGGACCACG GTCACCGTCT CCTCAGGTGG AGGCGGTTCA 480

GGCGGAGGTG GCTCTGGCGG TGGCGGATCG GACATCGAGC TCACTCAGTC TCCATTCTCC 540

CTGACTGTGA CAGCAGGAGA GAAGGTCACT ATGAATTGCA AGTCCGGTCA GAGTCTGTTA 600

AACAGTGTAA ATCAGAGGAA CTACTTGACC TGGTACCAGC AGAAGCCAGG GCAGCCTCCT 660

AAACTGTTGA TCTACTGGGC ATCCACTAGG GAATCTGGAG TCCCTGATCG CTTCACAGCC 720

AGTGGATCTG GAACAGATTT CACTCTCACC ATCAGCAGTG TGCAGGCTGA AGACCTGGCA 780

GTTTATTACT GTCAGAATGA TTATACTTAT CCGTTCACGT TCGGAGGGGG GACCAAGCTC 840

GAGATCAAAC GGGGATCCGG TAGCGGGAAC TCCGGTAAGG GGTACCTGAA GTAATAAGAT 900

CAAACGGTAA TAAGGATCCA GCTCGAATTC 930

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 156 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

CCATGGGATG GAGCTGTATC ATCCTCTTCT TGGTAGCAAC AGCTACAGGT AAGGGGCTCA 60

CAGTAGCAGG CTTGAGGTCT GGACATATAT ATGGGTGACA ATGACATCCA CTTTGCCTTT 120

CTCTCCACAG GTGTCCACTC CCAGGTCCAA CTGCAG 156

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

AGGTSMAMCT GCAGSAGTCW GG 22

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 32 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

TGAGGAGACG GTGACCGTGG TCCCTTGGCC CC 32

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

GACATTGAGC TCACCCAGTC TCCA 24

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

GTTAGATCTC GAGCTTGGTC CC 22

(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 45 base pairs

(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

CAGGATCCGG CCGGTTCGGC CCAGGTCCAG CTGCAACAGT CAGGA 45

(2) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 53 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

CTACATGAAT TCGCTAGCTT ATTATGAGGA GACGGTGACG GTGGTCCCTT GGC 53

(2) INFORMATION FOR SEQ ID NO: 11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 39 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

ATTGGAGTCG ACATCGAACT CACTCAGTCT CCATTCTCC 39

(2) INFORMATION FOR SEQ ID NO: 12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 36 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

CGAATTCGGA TCCCCGTTTG ATTTCGAGCT TGGTCC 36

(2) INFORMATION FOR SEQ ID NO: 13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 45 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

GAGCGCGAGC TCGGCCGAAC CGGCCGATCC GCCACCGCCA GAGCC 45

(2) INFORMATION FOR SEQ ID NO: 14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 36 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

ATTGTCGAAT TCGTCGACTC CGCCACCGCC AGAGCC 36

(2) INFORMATION FOR SEQ ID NO: 15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 57 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:

AGCTTCTAGA CCACCATGGA AAACTGCAGA GCTCAAAAGC TAGCGCGGCG GCTCTAG 57

(2) INFORMATION FOR SEQ ID NO: 16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 57 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

AATTCTAGAG CGGCCGCGCT AGCTTTTGAG CTCTGCAGTT TTCCATGGTG GTCTAGA 57

(2) INFORMATION FOR SEQ ID NO: 17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 40 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:

ACGGGTGAGC TCGATGTCGG AGTGGACACC TGTGGAGAGA 40

(2) INFORMATION FOR SEQ ID NO: 18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18:

GGAAACAGCT ATGACCATGA TTAC 24

(2) INFORMATION FOR SEQ ID NO: 19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "synthetic DNA"

(vii) IMMEDIATE SOURCE:
(B) CLONE: primer DBL.7

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 19:

CACCATCTCC AGAGACAATG GCAAG 25

(2) INFORMATION FOR SEQ ID NO: 20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "synthetic DNA"

(vii) IMMEDIATE SOURCE:
(B) CLONE: primer DBL.8

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 20:

ACCAAGCTCG AGATCAAACG GGG 23

(2) INFORMATION FOR SEQ ID NO: 21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 50 base pairs

(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "synthetic DNA"

(vii) IMMEDIATE SOURCE:
(B) CLONE: primer DBL.9

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 21:

TGAAGTGAAT TCGCGGCCGC TTATTACCGT TTGATTTCGA GCTTGGTCCC 50

(2) INFORMATION FOR SEQ ID NO: 22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 36 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "synthetic DNA"

(vii) IMMEDIATE SOURCE:
(B) CLONE: primer DBL.10

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 22:

TAATAAGCTA GCGGAGCTGC ATGCAAATTC TATTTC 36

(2) INFORMATION FOR SEQ ID NO: 23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 737 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "cDNA domains with synthetic linker(s)"

(vii) IMMEDIATE SOURCE:

(B) CLONE: EcoRI-HindIII insert of pUR4124

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION:11..730
(D) OTHER INFORMATION:/product= "VLlys-GS-VHlys"

(ix) FEATURE:
(A) NAME/KEY: mat_peptide
(B) LOCATION:11..334
(D) OTHER INFORMATION:/product= "VLlys"

(ix) FEATURE:
(A) NAME/KEY: misc_RNA
(B) LOCATION:335..379
(D) OTHER INFORMATION:/product= "(Gly4Ser)3 linker"

(ix) FEATURE:
(A) NAME/KEY: mat_peptide
(B) LOCATION:380..727
(D) OTHER INFORMATION:/product= "VHlys"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 23:

```
GAATTCGGCC GAC ATC GAG CTC ACC CAG TCT CCA GCC TCC CTT TCT GCG          49
          Asp Ile Glu Leu Thr Gln Ser Pro Ala Ser Leu Ser Ala
            1               5                  10

TCT GTG GGA GAA ACT GTC ACC ATC ACA TGT CGA GCA AGT GGG AAT ATT         97
Ser Val Gly Glu Thr Val Thr Ile Thr Cys Arg Ala Ser Gly Asn Ile
     15                  20                  25

CAC AAT TAT TTA GCA TGG TAT CAG CAG AAA CAG GGA AAA TCT CCT CAG        145
His Asn Tyr Leu Ala Trp Tyr Gln Gln Lys Gln Gly Lys Ser Pro Gln
 30                  35                  40                  45

CTC CTG GTC TAT TAT ACA ACA ACC TTA GCA GAT GGT GTG CCA TCA AGG        193
Leu Leu Val Tyr Tyr Thr Thr Thr Leu Ala Asp Gly Val Pro Ser Arg
                 50                  55                  60

TTC AGT GGC AGT GGA TCA GGA ACA CAA TAT TCT CTC AAG ATC AAC AGC        241
Phe Ser Gly Ser Gly Ser Gly Thr Gln Tyr Ser Leu Lys Ile Asn Ser
            65                  70                  75
```

```
CTG CAA CCT GAA GAT TTT GGG AGT TAT TAC TGT CAA CAT TTT TGG AGT      289
Leu Gln Pro Glu Asp Phe Gly Ser Tyr Tyr Cys Gln His Phe Trp Ser
        80                  85                  90

ACT CCT CGG ACG TTC GGT GGA GGG ACC AAG CTC GAG ATC AAA CGG GGT      337
Thr Pro Arg Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Arg Gly
     95                 100                 105

GGA GGC GGT TCA GGC GGA GGT GGC TCT GGC GGT GGC GGA TCG CAG GTG      385
Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gln Val
110                 115                 120                 125

CAG CTG CAG GAG TCA GGA CCT GGC CTG GTG GCG CCC TCA CAG AGC CTG      433
Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Ala Pro Ser Gln Ser Leu
                130                 135                 140

TCC ATC ACA TGC ACC GTC TCA GGG TTC TCA TTA ACC GGC TAT GGT GTA      481
Ser Ile Thr Cys Thr Val Ser Gly Phe Ser Leu Thr Gly Tyr Gly Val
            145                 150                 155


AAC TGG GTT CGC CAG CCT CCA GGA AAG GGT CTG GAG TGG CTG GGA ATG      529
Asn Trp Val Arg Gln Pro Pro Gly Lys Gly Leu Glu Trp Leu Gly Met
        160                 165                 170

ATT TGG GGT GAT GGA AAC ACA GAC TAT AAT TCA GCT CTC AAA TCC AGA      577
Ile Trp Gly Asp Gly Asn Thr Asp Tyr Asn Ser Ala Leu Lys Ser Arg
    175                 180                 185

CTG AGC ATC AGC AAG GAC AAC TCC AAG AGC CAA GTT TTC TTA AAA ATG      625
Leu Ser Ile Ser Lys Asp Asn Ser Lys Ser Gln Val Phe Leu Lys Met
190                 195                 200                 205

AAC AGT CTG CAC ACT GAT GAC ACA GCC AGG TAC TAC TGT GCC AGA GAG      673
Asn Ser Leu His Thr Asp Asp Thr Ala Arg Tyr Tyr Cys Ala Arg Glu
                210                 215                 220

AGA GAT TAT AGG CTT GAC TAC TGG GGC CAA GGG ACC ACG GTC ACC GTC      721
Arg Asp Tyr Arg Leu Asp Tyr Trp Gly Gln Gly Thr Thr Val Thr Val
            225                 230                 235

TCC TCA TGA TAAGCTT                                                 737
Ser Ser  *
```

240

(2) INFORMATION FOR SEQ ID NO: 24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 920 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "cDNA domains with synthetic linker(s)"

(vii) IMMEDIATE SOURCE:
(B) CLONE: HindIII-EcoRI insert Fv.3418

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION:36..443
(D) OTHER INFORMATION:/product= "pelB-VH3418"

(ix) FEATURE:
(A) NAME/KEY: sig_peptide
(B) LOCATION:36..101
(D) OTHER INFORMATION:/product= "pectate lyase"

(ix) FEATURE:
(A) NAME/KEY: mat_peptide
(B) LOCATION:102..440
(D) OTHER INFORMATION:/product= "VH3418"

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION:495..884
(D) OTHER INFORMATION:/product= "pelB-VL4318"

(ix) FEATURE:
(A) NAME/KEY: sig_peptide
(B) LOCATION:495..560
(D) OTHER INFORMATION:/product= "pectate lyase"

(ix) FEATURE:
(A) NAME/KEY: mat_peptide
(B) LOCATION:561..881

(D) OTHER INFORMATION:/product= "VL3418"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 24:

```
AAGCTTGCAA ATTCTATTTC AAGGAGACAG TCATA ATG AAA TAC CTA TTG CCT          53
                                       Met Lys Tyr Leu Leu Pro
                                      -22     -20

ACG GCA GCC GCT GGA TTG TTA TTA CTC GCT GCC CAA CCA GCG ATG GCC         101
Thr Ala Ala Ala Gly Leu Leu Leu Leu Ala Ala Gln Pro Ala Met Ala
    -15                 -10                 -5

CAG GTG CAG CTG CAG CAG TCA GGA CCT GAG CTG GTA AAG CCT GGG GCT         149
Gln Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Lys Pro Gly Ala
  1               5                  10                  15

TCA GTG AAG ATG TCC TGC AAG GCT TCT GGA TAC ACA TTC ACT AGC TAT         197
Ser Val Lys Met Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr
            20                  25                  30

GTT ATG CAC TGG GTG AAA CAG AAG CCT GGG CAG GGC CTT GAG TGG ATT         245
Val Met His Trp Val Lys Gln Lys Pro Gly Gln Gly Leu Glu Trp Ile
        35                  40                  45

GGA TAT ATT TAT CCT TAC AAT GAT GGT ACT AAG TAC AAT GAG AAG TTC         293
Gly Tyr Ile Tyr Pro Tyr Asn Asp Gly Thr Lys Tyr Asn Glu Lys Phe
    50                  55                  60

AAA GGC AAG GCC ACA CTG ACT TCA GAC AAA TCC TCC AGC ACA GCC TAC         341
Lys Gly Lys Ala Thr Leu Thr Ser Asp Lys Ser Ser Ser Thr Ala Tyr
65                  70                  75                  80

ATG GAG CTC AGC AGC CTG ACC TCT GAG GAC TCT GCG GTC TAT TAC TGT         389
Met Glu Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
                85                  90                  95

TCA AGA CGC TTT GAC TAC TGG GGC CAA GGG ACC ACG GTC ACC GTC TCC         437
Ser Arg Arg Phe Asp Tyr Trp Gly Gln Gly Thr Thr Val Thr Val Ser
            100                 105                 110

TCA TAA TAAGAGCTAT GGGAGCTTGC ATGCAAATTC TATTTCAAGG AGACAGTCAT         493
Ser  *

A ATG AAA TAC CTA TTG CCT ACG GCA GCC GCT GGA TTG TTA TTA CTC          539
```

```
  Met Lys Tyr Leu Leu Pro Thr Ala Ala Ala Gly Leu Leu Leu Leu
  -22      -20                 -15                 -10

GCT GCC CAA CCA GCG ATG GCC GAC ATC GAG CTC ACC CAG TCT CCA TCT       587
Ala Ala Gln Pro Ala Met Ala Asp Ile Glu Leu Thr Gln Ser Pro Ser
          -5                  1               5

TCC ATG TAT GCA TCT CTA GGA GAG AGA ATC ACT ATC ACT TGC AAG GCG       635
Ser Met Tyr Ala Ser Leu Gly Glu Arg Ile Thr Ile Thr Cys Lys Ala
 10                  15                  20                  25

AGT CAG GAC ATT AAT ACC TAT TTA ACC TGG TTC CAG CAG AAA CCA GGG       683
Ser Gln Asp Ile Asn Thr Tyr Leu Thr Trp Phe Gln Gln Lys Pro Gly
                 30                  35                  40

AAA TCT CCC AAG ACC CTG ATC TAT CGT GCA AAC AGA TTG CTA GAT GGG       731
Lys Ser Pro Lys Thr Leu Ile Tyr Arg Ala Asn Arg Leu Leu Asp Gly
             45                  50                  55

GTC CCA TCA AGG TTC AGT GGC AGT GGA TCT GGG CAA GAT TAT TCT CTC       779
Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Gln Asp Tyr Ser Leu
         60                  65                  70

ACC ATC AGC AGC CTG GAC TAT GAA GAT ATG GGA ATT TAT TAT TGT CTA       827
Thr Ile Ser Ser Leu Asp Tyr Glu Asp Met Gly Ile Tyr Tyr Cys Leu
     75                  80                  85

CAA TAT GAT GAG TTG TAC ACG TTC GGA GGG GGG ACC AAG CTC GAG ATC       875
Gln Tyr Asp Glu Leu Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile
 90                  95                  100                 105

AAA CGG TAA TAATGATCAA ACGGTATAAG GATCCAGCTC GAATTC                   920
Lys Arg  *
```

(2) INFORMATION FOR SEQ ID NO: 25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 999 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "cDNA domains with synthetic linker(s)"

(vii) IMMEDIATE SOURCE:
(B) CLONE: HindIII-EcoRI insert of Fv.4715-myc

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION:40..468
(D) OTHER INFORMATION:/product= "pelB-VH4715"

(ix) FEATURE:
(A) NAME/KEY: sig_peptide
(B) LOCATION:40..105
(D) OTHER INFORMATION:/product= "pectate lyase"

(ix) FEATURE:
(A) NAME/KEY: mat_peptide
(B) LOCATION:106..465
(D) OTHER INFORMATION:/product= "VH4715"

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION:520..963
(D) OTHER INFORMATION:/product= "pelB-VL4715-myc"

(ix) FEATURE:
(A) NAME/KEY: sig_peptide
(B) LOCATION:520..585
(D) OTHER INFORMATION:/product= "pectate lyase"

(ix) FEATURE:
(A) NAME/KEY: mat_peptide
(B) LOCATION:586..927
(D) OTHER INFORMATION:/product= "VL4715"

(ix) FEATURE:
(A) NAME/KEY: misc_RNA
(B) LOCATION:928..960
(D) OTHER INFORMATION:/product= "myc-tag"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 25:

```
AAGCTTGCAT GCAAATTCTA TTTCAAGGAG ACAGTCATA ATG AAA TAC CTA TTG          54
                                           Met Lys Tyr Leu Leu
                                           -22     -20
```

```
CCT ACG GCA GCC GCT GGA TTG TTA TTA CTC GCT GCC CAA CCA GCG ATG       102
Pro Thr Ala Ala Ala Gly Leu Leu Leu Leu Ala Ala Gln Pro Ala Met
        -15                 -10                 -5

GCC CAG GTG CAG CTG CAG GAG TCA GGG GGA GAC TTA GTG AAG CCT GGA       150
Ala Gln Val Gln Leu Gln Glu Ser Gly Gly Asp Leu Val Lys Pro Gly
      1               5                  10                  15

GGG TCC CTG ACA CTC TCC TGT GCA ACC TCT GGA TTC ACT TTC AGT AGT       198
Gly Ser Leu Thr Leu Ser Cys Ala Thr Ser Gly Phe Thr Phe Ser Ser
                  20                  25                  30

TAT GCC TTT TCT TGG GTC CGC CAG ACC TCA GAC AAG AGT CTG GAG TGG       246
Tyr Ala Phe Ser Trp Val Arg Gln Thr Ser Asp Lys Ser Leu Glu Trp
              35                  40                  45

GTC GCA ACC ATC AGT AGT ACT GAT ACT TAT ACC TAT TAT TCA GAC AAT       294
Val Ala Thr Ile Ser Ser Thr Asp Thr Tyr Thr Tyr Tyr Ser Asp Asn
          50                  55                  60

GTG AAG GGG CGC TTC ACC ATC TCC AGA GAC AAT GGC AAG AAC ACC CTG       342
Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Gly Lys Asn Thr Leu
      65                  70                  75

TAC CTG CAA ATG AGC AGT CTG AAG TCT GAG GAC ACA GCC GTG TAT TAC       390
Tyr Leu Gln Met Ser Ser Leu Lys Ser Glu Asp Thr Ala Val Tyr Tyr
  80                  85                  90                  95

TGT GCA AGA CAT GGG TAC TAT GGT AAA GGC TAT TTT GAC TAC TGG GGC       438
Cys Ala Arg His Gly Tyr Tyr Gly Lys Gly Tyr Phe Asp Tyr Trp Gly
                 100                 105                 110

CAA GGG ACC ACG GTC ACC GTC TCC TCA TAA TAAGAGCTAT GGGAGCTTGC        488
Gln Gly Thr Thr Val Thr Val Ser Ser  *
             115                 120

ATGCAAATTC TATTTCAAGG AGACAGTCAT A ATG AAA TAC CTA TTG CCT ACG       540
                                   Met Lys Tyr Leu Leu Pro Thr
                                   -22     -20

GCA GCC GCT GGA TTG TTA TTA CTC GCT GCC CAA CCA GCG ATG GCC GAC       588
Ala Ala Ala Gly Leu Leu Leu Leu Ala Ala Gln Pro Ala Met Ala Asp
-15                 -10                 -5                  1
```

```
ATC GAG CTC ACT CAG TCT CCA TTC TCC CTG ACT GTG ACA GCA GGA GAG      636
Ile Glu Leu Thr Gln Ser Pro Phe Ser Leu Thr Val Thr Ala Gly Glu
                5                   10                  15

AAG GTC ACT ATG AAT TGC AAG TCC GGT CAG AGT CTG TTA AAC AGT GTA      684
Lys Val Thr Met Asn Cys Lys Ser Gly Gln Ser Leu Leu Asn Ser Val
            20                  25                  30

AAT CAG AGG AAC TAC TTG ACC TGG TAC CAG CAG AAG CCA GGG CAG CCT      732
Asn Gln Arg Asn Tyr Leu Thr Trp Tyr Gln Gln Lys Pro Gly Gln Pro
        35                  40                  45

CCT AAA CTG TTG ATC TAC TGG GCA TCC ACT AGG GAA TCT GGA GTC CCT      780
Pro Lys Leu Leu Ile Tyr Trp Ala Ser Thr Arg Glu Ser Gly Val Pro
    50                  55                  60                  65

GAT CGC TTC ACA GCC AGT GGA TCT GGA ACA GAT TTC ACT CTC ACC ATC      828
Asp Arg Phe Thr Ala Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile
                70                  75                  80

AGC AGT GTG CAG GCT GAA GAC CTG GCA GTT TAT TAC TGT CAG AAT GAT      876
Ser Ser Val Gln Ala Glu Asp Leu Ala Val Tyr Tyr Cys Gln Asn Asp
            85                  90                  95

TAT ACT TAT CCG TTC ACG TTC GGA GGG GGG ACC AAG CTC GAG ATC AAA      924
Tyr Thr Tyr Pro Phe Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
        100                 105                 110

CGG GAA CAA AAA CTC ATC TCA GAA GAG GAT CTG AAT TAA TAAGATCAAA       973
Arg Glu Gln Lys Leu Ile Ser Glu Glu Asp Leu Asn  *
    115                 120                 125

CGGTAATAAG GATCCAGCTC GAATTC                                        999
```

(2) INFORMATION FOR SEQ ID NO: 26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 924 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "cDNA domains with synthetic linker(s)"

(vii) IMMEDIATE SOURCE:
(B) CLONE: HindIII-EcoRI insert of scFv.4715-myc

(ix) FEATURE:
(A) NAME/KEY: sig_peptide
(B) LOCATION:40..105
(D) OTHER INFORMATION:/product= "pectate lyase"

(ix) FEATURE:
(A) NAME/KEY: mat_peptide
(B) LOCATION:106..465
(D) OTHER INFORMATION:/product= "VH4715"

(ix) FEATURE:
(A) NAME/KEY: misc_RNA
(B) LOCATION:466..510
(D) OTHER INFORMATION:/product= "(Gly4Ser)3-linker"

(ix) FEATURE:
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(B) LOCATION:511..852
(D) OTHER INFORMATION:/product= "VL4715"

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(B) LOCATION:853..885
(D) OTHER INFORMATION:/product= "myc-tag"

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION:40..888
(D) OTHER INFORMATION:/product=
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(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 26:

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                                          -22     -20

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  1               5                  10                  15

GGG TCC CTG ACA CTC TCC TGT GCA ACC TCT GGA TTC ACT TTC AGT AGT      198
Gly Ser Leu Thr Leu Ser Cys Ala Thr Ser Gly Phe Thr Phe Ser Ser
            20                  25                  30

TAT GCC TTT TCT TGG GTC CGC CAG ACC TCA GAC AAG AGT CTG GAG TGG      246
Tyr Ala Phe Ser Trp Val Arg Gln Thr Ser Asp Lys Ser Leu Glu Trp
        35                  40                  45

GTC GCA ACC ATC AGT AGT ACT GAT ACT TAT ACC TAT TAT TCA GAC AAT      294
Val Ala Thr Ile Ser Ser Thr Asp Thr Tyr Thr Tyr Tyr Ser Asp Asn
    50                  55                  60

GTG AAG GGG CGC TTC ACC ATC TCC AGA GAC AAT GGC AAG AAC ACC CTG      342
Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Gly Lys Asn Thr Leu
65                  70                  75

TAC CTG CAA ATG AGC AGT CTG AAG TCT GAG GAC ACA GCC GTG TAT TAC      390
Tyr Leu Gln Met Ser Ser Leu Lys Ser Glu Asp Thr Ala Val Tyr Tyr
80                  85                  90                  95

TGT GCA AGA CAT GGG TAC TAT GGT AAA GGC TAT TTT GAC TAC TGG GGC      438
Cys Ala Arg His Gly Tyr Tyr Gly Lys Gly Tyr Phe Asp Tyr Trp Gly
            100                 105                 110

CAA GGG ACC ACG GTC ACC GTC TCC TCA GGT GGA GGC GGT TCA GGC GGA      486
Gln Gly Thr Thr Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly
        115                 120                 125

GGT GGC TCT GGC GGT GGC GGA TCG GAC ATC GAG CTC ACT CAG TCT CCA      534
Gly Gly Ser Gly Gly Gly Gly Ser Asp Ile Glu Leu Thr Gln Ser Pro
    130                 135                 140

TTC TCC CTG ACT GTG ACA GCA GGA GAG AAG GTC ACT ATG AAT TGC AAG      582
Phe Ser Leu Thr Val Thr Ala Gly Glu Lys Val Thr Met Asn Cys Lys
145                 150                 155

TCC GGT CAG AGT CTG TTA AAC AGT GTA AAT CAG AGG AAC TAC TTG ACC      630
Ser Gly Gln Ser Leu Leu Asn Ser Val Asn Gln Arg Asn Tyr Leu Thr
160                 165                 170                 175
```

```
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Trp Tyr Gln Gln Lys Pro Gly Gln Pro Pro Lys Leu Leu Ile Tyr Trp
                180                 185                 190

GCA TCC ACT AGG GAA TCT GGA GTC CCT GAT CGC TTC ACA GCC AGT GGA       726
Ala Ser Thr Arg Glu Ser Gly Val Pro Asp Arg Phe Thr Ala Ser Gly
            195                 200                 205

TCT GGA ACA GAT TTC ACT CTC ACC ATC AGC AGT GTG CAG GCT GAA GAC       774
Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Val Gln Ala Glu Asp
        210                 215                 220

CTG GCA GTT TAT TAC TGT CAG AAT GAT TAT ACT TAT CCG TTC ACG TTC       822
Leu Ala Val Tyr Tyr Cys Gln Asn Asp Tyr Thr Tyr Pro Phe Thr Phe
    225                 230                 235

GGA GGG GGG ACC AAG CTC GAG ATC AAA CGG GAA CAA AAA CTC ATC TCA       870
Gly Gly Gly Thr Lys Leu Glu Ile Lys Arg Glu Gln Lys Leu Ile Ser
240                 245                 250                 255

GAA GAG GAT CTG AAT TAA TAAGATCAAA CGGTAATAAG GATCCAGCTC GAATTC       924
Glu Glu Asp Leu Asn  *
                260
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(2) INFORMATION FOR SEQ ID NO: 27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1706 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "cDNA domains with synthetic linker(s)"

(vii) IMMEDIATE SOURCE:
(B) CLONE: HindIII-EcoRI insert of pGOSA.E

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION:40..864
(D) OTHER INFORMATION:/product= "pelB-VH4715-LiA-VH3418"

(ix) FEATURE:

(A) NAME/KEY: sig_peptide
(B) LOCATION:40..105
(D) OTHER INFORMATION:/product= "pectate lyase"

(ix) FEATURE:
(A) NAME/KEY: mat_peptide
(B) LOCATION:106..465
(D) OTHER INFORMATION:/product= "VH4715"

(ix) FEATURE:
(A) NAME/KEY: misc_RNA
(B) LOCATION:466..522
(D) OTHER INFORMATION:/product= "linkerA
(Gly4Ser)3AlaGlySerAla"

(ix) FEATURE:
(A) NAME/KEY: mat_peptide
(B) LOCATION:523..861
(D) OTHER INFORMATION:/product= "VH3418"

(ix) FEATURE:
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(B) LOCATION:913..1689
(D) OTHER INFORMATION:/product= "pelB-VL3418-LiV-VL4715"

(ix) FEATURE:
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(B) LOCATION:913..978
(D) OTHER INFORMATION:/product= "pectate lyase"

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(B) LOCATION:979..1299
(D) OTHER INFORMATION:/product= "VL3418"

(ix) FEATURE:
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(B) LOCATION:1300..1344
(D) OTHER INFORMATION:/product= "linker V
(Gly4Ser)2Gly4Val"

(ix) FEATURE:
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(B) LOCATION:1345..1686

(D) OTHER INFORMATION:/product= "VL4715"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 27:

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                                            Met Lys Tyr Leu Leu
                                           -22  -20

CCT ACG GCA GCC GCT GGA TTG TTA TTA CTC GCT GCC CAA CCA GCG ATG         102
Pro Thr Ala Ala Ala Gly Leu Leu Leu Leu Ala Ala Gln Pro Ala Met
        -15                 -10                 -5

GCC CAG GTG CAG CTG CAG GAG TCA GGG GGA GAC TTA GTG AAG CCT GGA         150
Ala Gln Val Gln Leu Gln Glu Ser Gly Gly Asp Leu Val Lys Pro Gly
      1               5                  10                  15

GGG TCC CTG ACA CTC TCC TGT GCA ACC TCT GGA TTC ACT TTC AGT AGT         198
Gly Ser Leu Thr Leu Ser Cys Ala Thr Ser Gly Phe Thr Phe Ser Ser
                20                  25                  30

TAT GCC TTT TCT TGG GTC CGC CAG ACC TCA GAC AAG AGT CTG GAG TGG         246
Tyr Ala Phe Ser Trp Val Arg Gln Thr Ser Asp Lys Ser Leu Glu Trp
             35                  40                  45

GTC GCA ACC ATC AGT AGT ACT GAT ACT TAT ACC TAT TAT TCA GAC AAT         294
Val Ala Thr Ile Ser Ser Thr Asp Thr Tyr Thr Tyr Tyr Ser Asp Asn
         50                  55                  60

GTG AAG GGG CGC TTC ACC ATC TCC AGA GAC AAT GGC AAG AAC ACC CTG         342
Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Gly Lys Asn Thr Leu
     65                  70                  75

TAC CTG CAA ATG AGC AGT CTG AAG TCT GAG GAC ACA GCC GTG TAT TAC         390
Tyr Leu Gln Met Ser Ser Leu Lys Ser Glu Asp Thr Ala Val Tyr Tyr
 80                  85                  90                  95

TGT GCA AGA CAT GGG TAC TAT GGT AAA GGC TAT TTT GAC TAC TGG GGC         438
Cys Ala Arg His Gly Tyr Tyr Gly Lys Gly Tyr Phe Asp Tyr Trp Gly
                100                 105                 110

CAA GGG ACC ACG GTC ACC GTC TCC TCA GGT GGA GGC GGT TCA GGC GGA         486
Gln Gly Thr Thr Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly
            115                 120                 125
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GGT GGC TCT GGC GGT GGC GGA TCG GCC GGT TCG GCC CAG GTC CAG CTG      534
Gly Gly Ser Gly Gly Gly Gly Ser Ala Gly Ser Ala Gln Val Gln Leu
        130                 135                 140

CAA CAG TCA GGA CCT GAG CTG GTA AAG CCT GGG GCT TCA GTG AAG ATG      582
Gln Gln Ser Gly Pro Glu Leu Val Lys Pro Gly Ala Ser Val Lys Met
    145                 150                 155

TCC TGC AAG GCT TCT GGA TAC ACA TTC ACT AGC TAT GTT ATG CAC TGG      630
Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr Val Met His Trp
160                 165                 170                 175

GTG AAA CAG AAG CCT GGG CAG GGC CTT GAG TGG ATT GGA TAT ATT TAT      678
Val Lys Gln Lys Pro Gly Gln Gly Leu Glu Trp Ile Gly Tyr Ile Tyr
                180                 185                 190

CCT TAC AAT GAT GGT ACT AAG TAC AAT GAG AAG TTC AAA GGC AAG GCC      726
Pro Tyr Asn Asp Gly Thr Lys Tyr Asn Glu Lys Phe Lys Gly Lys Ala
            195                 200                 205

ACA CTG ACT TCA GAC AAA TCC TCC AGC ACA GCC TAC ATG GAG CTC AGC      774
Thr Leu Thr Ser Asp Lys Ser Ser Ser Thr Ala Tyr Met Glu Leu Ser
        210                 215                 220

AGC CTG ACC TCT GAG GAC TCT GCG GTC TAT TAC TGT TCA AGA CGC TTT      822
Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys Ser Arg Arg Phe
    225                 230                 235

GAC TAC TGG GGC CAA GGG ACC ACC GTC ACC GTC TCC TCA TAA              864
Asp Tyr Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser  *
240                 245                 250

TAAGCTAGCG GAGCTGCATG CAAATTCTAT TTCAAGGAGA CAGTCATA ATG AAA TAC     921
                                                Met Lys Tyr
                                               -22     -20

CTA TTG CCT ACG GCA GCC GCT GGA TTG TTA TTA CTC GCT GCC CAA CCA      969
Leu Leu Pro Thr Ala Ala Ala Gly Leu Leu Leu Leu Ala Ala Gln Pro
                -15                 -10                 -5

GCG ATG GCC GAC ATC GAG CTC ACC CAG TCT CCA TCT TCC ATG TAT GCA     1017
Ala Met Ala Asp Ile Glu Leu Thr Gln Ser Pro Ser Ser Met Tyr Ala
            1               5                   10
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     15                  20                  25

AAT ACC TAT TTA ACC TGG TTC CAG CAG AAA CCA GGG AAA TCT CCC AAG      1113
Asn Thr Tyr Leu Thr Trp Phe Gln Gln Lys Pro Gly Lys Ser Pro Lys
 30                  35                  40                  45

ACC CTG ATC TAT CGT GCA AAC AGA TTG CTA GAT GGG GTC CCA TCA AGG      1161
Thr Leu Ile Tyr Arg Ala Asn Arg Leu Leu Asp Gly Val Pro Ser Arg
                 50                  55                  60

TTC AGT GGC AGT GGA TCT GGG CAA GAT TAT TCT CTC ACC ATC AGC AGC      1209
Phe Ser Gly Ser Gly Ser Gly Gln Asp Tyr Ser Leu Thr Ile Ser Ser
             65                  70                  75

CTG GAC TAT GAA GAT ATG GGA ATT TAT TAT TGT CTA CAA TAT GAT GAG      1257
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         80                  85                  90

TTG TAC ACG TTC GGA GGG GGG ACC AAG CTC GAG ATC AAA CGG GGT GGA      1305
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     95                  100                 105

GGC GGT TCA GGC GGA GGT GGC TCT GGC GGT GGC GGA GTC GAC ATC GAA      1353
Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Val Asp Ile Glu
110                 115                 120                 125

CTC ACT CAG TCT CCA TTC TCC CTG ACT GTG ACA GCA GGA GAG AAG GTC      1401
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                130                 135                 140

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190                 195                 200                 205

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Val Gln Ala Glu Asp Leu Ala Val Tyr Tyr Cys Gln Asn Asp Tyr Thr
                210                 215                 220

TAT CCG TTC ACG TTC GGA GGG GGG ACC AAG CTC GAA ATC AAA CGG TAA      1689
Tyr Pro Phe Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Arg  *
            225                 230                 235

TAAGCGGCCG CGAATTC                                                   1706
```

CLAIMS

1. A multivalent antigen binding protein comprising:
a first polypeptide comprising, in series, three or more variable domains of an antibody heavy chain; and
a second polypeptide comprising, in series, three or more variable domains of an antibody light chain,

said first and second polypeptides being linked by association of the respective heavy chain and light chain variable domains, each associated variable domain pair forming an antigen binding site.

2. A protein according to Claim 1 comprising a trivalent antigen binding protein.

3. A protein according to Claim 1 or Claim 2 wherein the variable domains of the antibody heavy chain of said first polypeptide are linked by a peptide linker and the variable domains of the antibody light chain of said second polypeptide are linked by a peptide linker.

4. A protein according to any one of Claims 1 to 3 wherein the associated variable domain pair binding sites are able to bind different epitopes from each other.

5. A protein according to any one of Claims 1 to 3 wherein the associated variable domain pair binding sites are able to bind the same epitope as each other.

6. Nucleotide sequences coding for the polypeptides of the multivalent antigen binding protein of any one

of the preceding claims.

7. Nucleotide sequences according to Claim 6 contained in one or more expression vectors.

8. A host cell transformed with a vector according to Claim 7, and capable of expression of the nucleotide sequences to produce the polypeptides of the multivalent antigen binding protein.

9. A host cell according to Claim 8 wherein the polypeptides on expression associate to form the multivalent antigen binding protein.

10. A process for preparing a multivalent antigen binding protein according to any one of Claims 1 to 5 comprising

(i) transforming one or more hosts by incorporating genes encoding said first and second polypeptides;
(ii) expressing said genes and said host or hosts; and
(iii) allowing said first and second polypeptides to associate to form the protein.

11. A protein according to any one of Claims 1 to 5 for use in medicine.

12. A diagnostic or therapeutic composition comprising a protein according to any one of Claims 1 to 5.

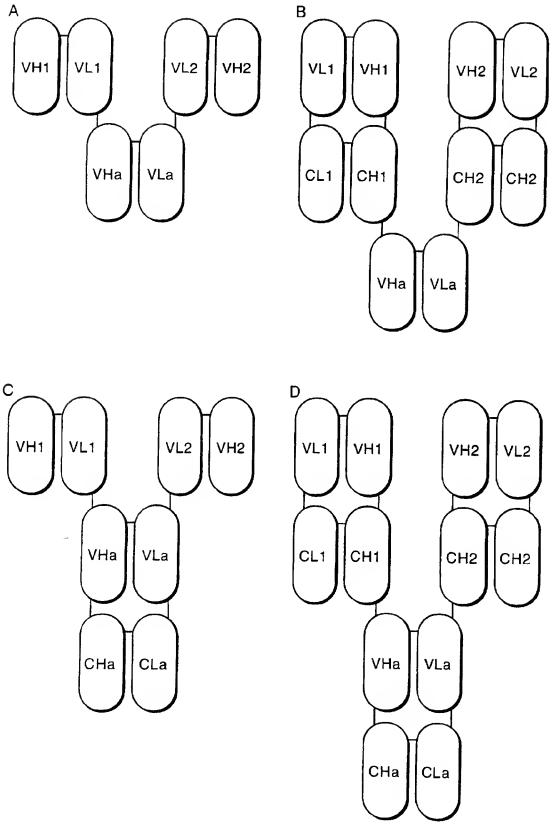
13. Use of composition according to Claim 12 in the preparation of an agent for use in diagnosis or therapy.

14. A method of diagnosis or therapy comprising administering a protein according to any one of

Claims 1 to 5.

15. Use of a protein according to any one of Claims 1 to 5 in an immunoassay method or for purification.

Fig.1A.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VHa
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CHa
CLa
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VHa
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CLa

Fig.1B.

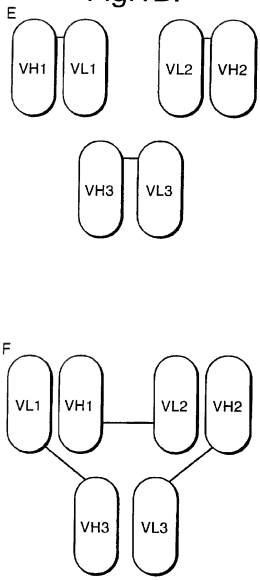
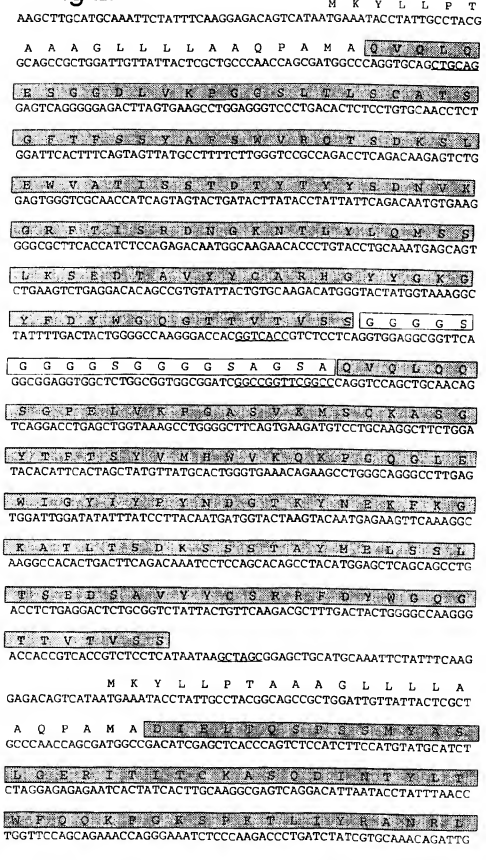


Fig.2A.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pelB leader
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GCAGCCGCTGGATTGTTATTACTCGCTGCCCAACCAGCGATGGCCCAGGTGCAGCTGCAG
E S G G D L V K P G G S L T L S C A T S
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G F T F S S Y A F S W V R Q T S D K S L
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L K S E D T A V Y Y C A R H G Y Y G K G
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TATTTTGACTACTGGGGCCAAGGGACCACGGTCACCGTCTCCTCAGGTGGAGGCGGTTCA
Linker
G G G G S G G G G S A G S A Q V Q L Q Q
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W I G Y I Y P Y N D G T K Y N E K F K G
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T T V T V S S
ACCACCGTCACCGTCTCCTCATAATAAGCTAGCGGAGCTGCATGCAAATTCTATTTCAAG
M K Y L L P T A A G L L L L A
pelB leader
GAGACAGTCATAATGAAATACCTATTGCCTACGGCAGCCGCTGGATTGTTATTACTCGCT
A Q P A M A D I E L T Q S P S S M Y A S
GCCCAACCAGCGATGGCCGACATCGAGCTCACCCAGTCTCCATCTTCCATGTATGCATCT
L G E R I T I T C K A S Q D I N T Y L T
VL3418
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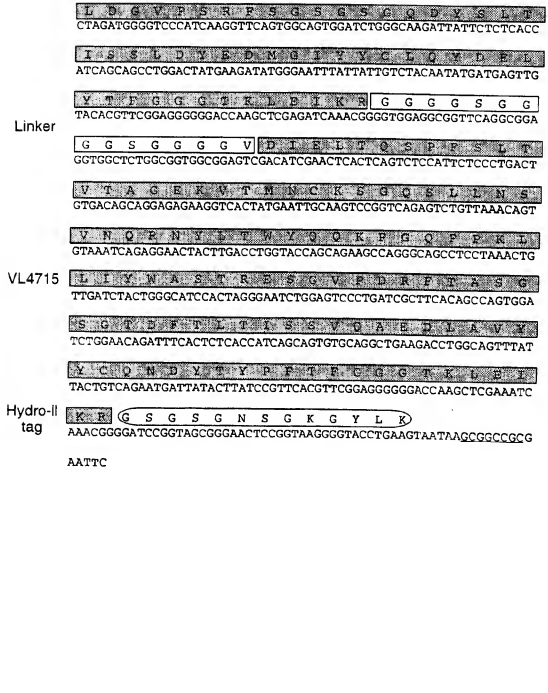
Fig.2B.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ATCAGCAGCCTGGACTATGAAGATATGGGAATTTATTATTGTCTACAATATGATGAGTTG
V T F G G G T K L E I K R G G G G S G G
TACACGTTCGGAGGGGGGACCAAGCTCGAGATCAAACGGGGTGGAGGCGGTTCAGGCGGA
Linker
G G S G G G G V D I E L T Q S P P S L T
GGTGGCTCTGGCGGTGGCGGAGTCGACATCGAACTCACTCAGTCTCCATTCTCCCTGACT
V T A G E K V T M N C K S S Q S L L N S
GTGACAGCAGGAGAGAAGGTCACTATGAATTGCAAGTCCGGTCAGAGTCTGTTAAACAGT
V N Q K N Y L T W Y Q Q K P G Q P P K L
GTAAATCAGAAGAACTACTTGACCTGGTACCAGCAGAAGCCAGGGCAGCCTCCTAAACTG
VL4715
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Y C Q N D Y T Y P F T F G G G T K L E I
TACTGTCAGAATGATTATACTTATCCGTTCACGTTCGGAGGGGGGACCAAGCTCGAAATC
Hydro-II tag
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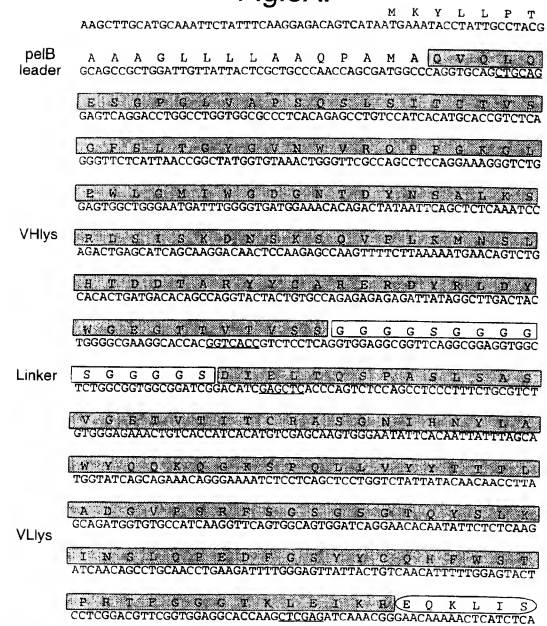
Fig.3A.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E W L G M I W G D G N T D Y N S A L K S
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VHlys
R L S I S K D N S K S Q V F L K M N S L
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H T D D T A R Y Y C A R E R D Y R L D Y
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W G E G T T V T V S S G G G G S G G G G
TGGGGCGAAGGCACCACGGTCACCGTCTCCTCAGGTGGAGGCGGTTCAGGCGGAGGTGGC
Linker
S G G G G S D I E L T Q S P A S L S A S
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V G E T V T I T C R A S G N I H N Y L A
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W Y Q Q K Q G K S P Q L L V Y Y T T T L
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A D G V P S R F S G S G S G T Q Y S L K
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VLlys
I N S L Q P E D F G S Y Y C Q H F W S T
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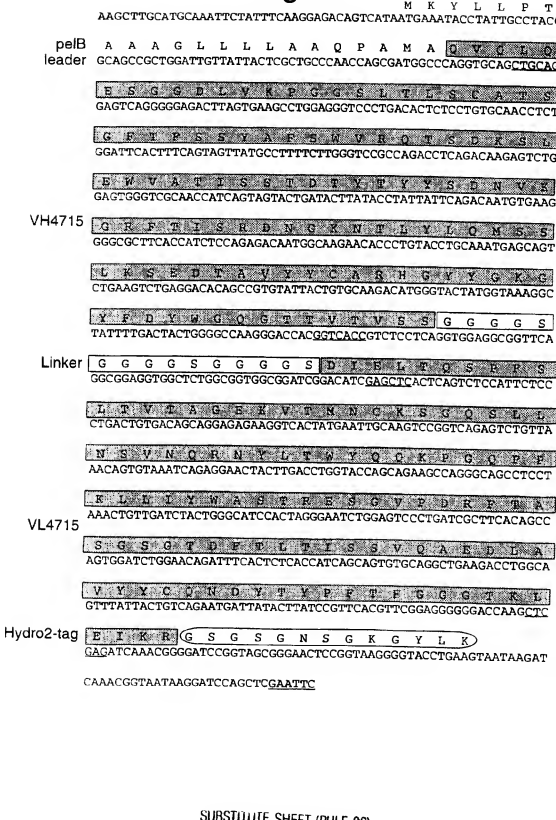
P R T F G G G T K L E I K R E Q K L I S
CCTCGGACGTTCGGTGGAGGCACCAAGCTCGAGATCAAACGGGAACAAAAACTCATCTCA
Myc-tag
E E D L N
GAAGAGGATCTGAATTAATAAGATCAAACGGTAATAAGGATCCAGCTCGAATTC

Fig.3B.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pelB leader
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E S G G D L V K P G G S L T L S C A T S
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G F T F S S Y A F S W V R Q T S D K S L
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E W V A T I S S G G D Y I Y Y S D N V K
GAGTGGGTCGCAACCATCAGTAGTACTGATACTTATACCTATTATTCAGACAATGTGAAG
VH4715
G R F T I S R D N G K N T L Y L Q M S S
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L K S E D T A V Y Y C A S H G Y Y G K G
CTGAAGTCTGAGGACACAGCCGTGTATTACTGTGCAAGACATGGGTACTATGGTAAAGGC
Y F D Y W G Q G T T V T V S S G G G G S
TATTTTGACTACTGGGGCCAAGGGACCACGGTCACCGTCTCCTCAGGTGGAGGCGGTTCA
Linker
G G G G S G G G G S D Y E L T Q S P R S
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L T V T A G E K V T M N C K S S Q S L L
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N S V N Q R N Y L T W Y Q Q K P G Q P P
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K L L I Y W A S T R E S G V P D R F T G
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VL4715
S G S G T D F T L T I S S V Q A E D L A
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V Y Y C Q N D Y T Y P F T F G G G T K L
GTTTATTACTGTCAGAATGATTATACTTATCCGTTCACGTTCGGAGGGGGGACCAAGCTC
Hydro2-tag
E I K R G S G S G N S G K G Y L K
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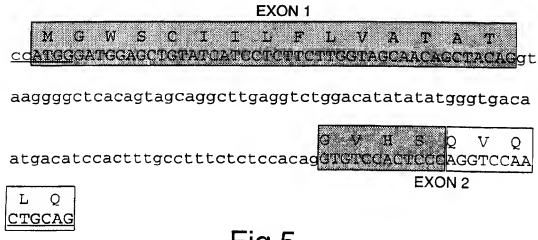
Fig.4.


EXON 1
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atgacatccactttgcctttctctccacagGTGTCCACTCCCAGGTCCAA
EXON 2
L Q
CTGCAG

Fig.5.

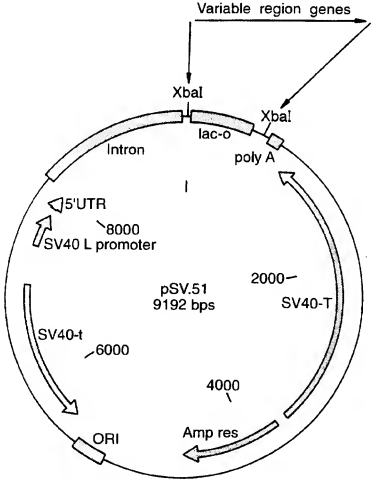
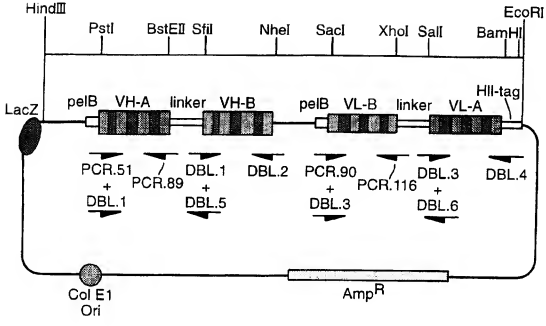

Variable region genes
XbaI
XbaI
lac-o
Intron
poly A
5'UTR
8000
SV40 L promoter
2000
pSV.51
9192 bps
SV40-T
SV40-t
6000
4000
ORI
Amp res

Fig.6A.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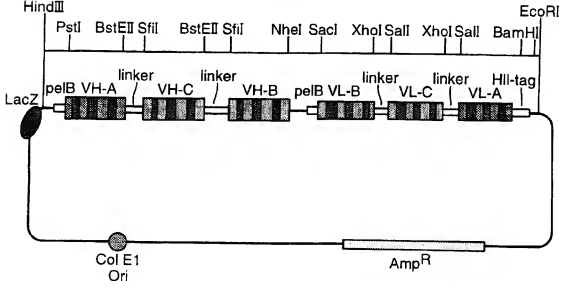
EcoRI
PstI
BstEII SfiI
NheI
SacI
XhoI
SalI
BamHI
LacZ
pelB
VH-A
linker
VH-B
pelB
VL-B
linker
VL-A
HII-tag
PCR.51
+
DBL.1
PCR.89
DBL.1
+
DBL.5
DBL.2
PCR.90
+
DBL.3
PCR.116
DBL.3
+
DBL.6
DBL.4
Col E1
Ori
AmpR

Fig.6B.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PstI
BstEII SfiI
BstEII SfiI
NheI
SacI
XhoI
SalI
XhoI
SalI
BamHI
LacZ
pelB
VH-A
linker
VH-C
linker
VH-B
pelB
VL-B
linker
VL-C
linker
VL-A
HII-tag
Col E1
Ori
AmpR

Fig.7A.

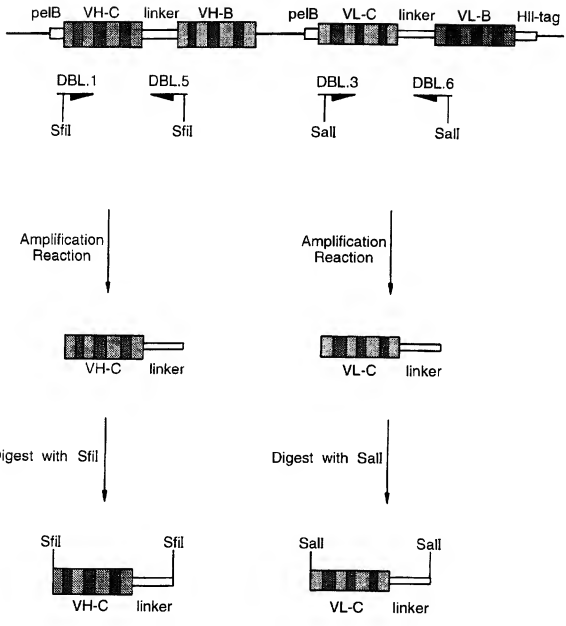

pelB
VH-C
linker
VH-B
pelB
VL-C
linker
VL-B
Hll-tag
DBL.1
DBL.5
DBL.3
DBL.6
SfiI
SfiI
SalI
SalI
Amplification Reaction
Amplification Reaction
VH-C
linker
VL-C
linker
Digest with SfiI
Digest with SalI
SfiI
SfiI
SalI
SalI
VH-C
linker
VL-C
linker

Fig.7B.

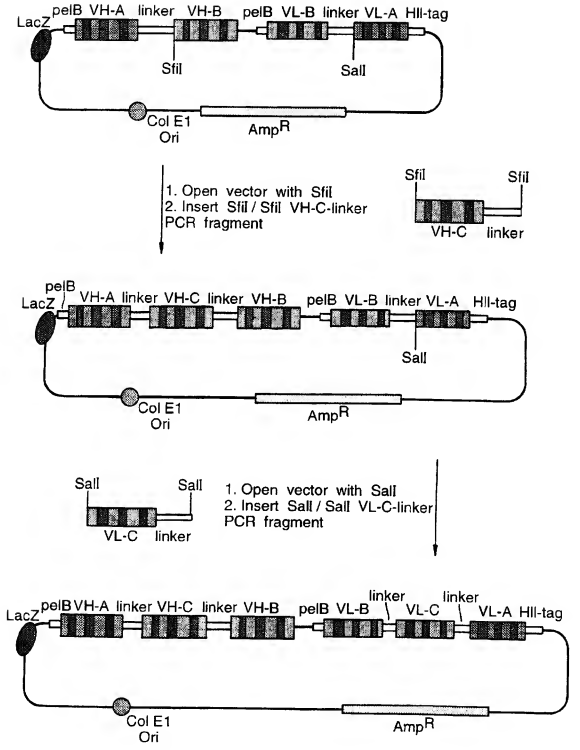

LacZ
pelB VH-A linker VH-B pelB VL-B linker VL-A HII-tag
SfiI
SalI
Col E1 Ori
AmpR
1. Open vector with SfiI
2. Insert SfiI / SfiI VH-C-linker PCR fragment
SfiI
SfiI
VH-C
linker
pelB
LacZ
VH-A linker VH-C linker VH-B pelB VL-B linker VL-A HII-tag
SalI
Col E1 Ori
AmpR
SalI
SalI
VL-C
linker
1. Open vector with SalI
2. Insert SalI / SalI VL-C-linker PCR fragment
linker
linker
LacZ
pelB VH-A linker VH-C linker VH-B pelB VL-B VL-C VL-A HII-tag
Col E1 Ori
AmpR

Fig.8A.

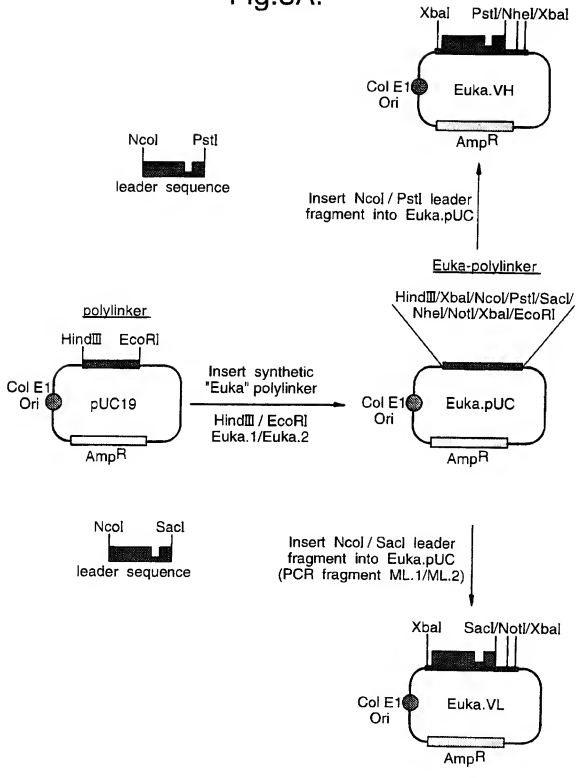

XbaI
PstI/NheI/XbaI
Col E1 Ori
Euka.VH
AmpR
NcoI
PstI
leader sequence
Insert NcoI / PstI leader fragment into Euka.pUC
Euka-polylinker
HindIII/XbaI/NcoI/PstI/SacI/ NheI/NotI/XbaI/EcoRI
polylinker
HindIII
EcoRI
Col E1 Ori
pUC19
AmpR
Insert synthetic "Euka" polylinker
HindIII / EcoRI Euka.1/Euka.2
Col E1 Ori
Euka.pUC
AmpR
NcoI
SacI
leader sequence
Insert NcoI / SacI leader fragment into Euka.pUC (PCR fragment ML.1/ML.2)
XbaI
SacI/NotI/XbaI
Col E1 Ori
Euka.VL
AmpR

Fig.8B.

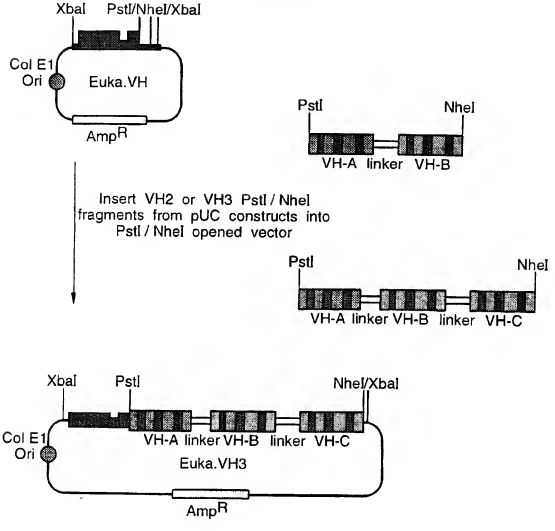

XbaI
PstI/NheI/XbaI
Col E1 Ori
Euka.VH
AmpR
PstI
NheI
VH-A linker VH-B
Insert VH2 or VH3 PstI / NheI fragments from pUC constructs into PstI / NheI opened vector
PstI
NheI
VH-A linker VH-B linker VH-C
XbaI
PstI
NheI/XbaI
Col E1 Ori
VH-A linker VH-B linker VH-C
Euka.VH3
AmpR
Excise XbaI / XbaI fragments from Euka constructs and insert into XbaI opened pSV.51 vector
pSV.B/pSV.D/pSV.G/pSV.K/pSV.M

Fig.8C.

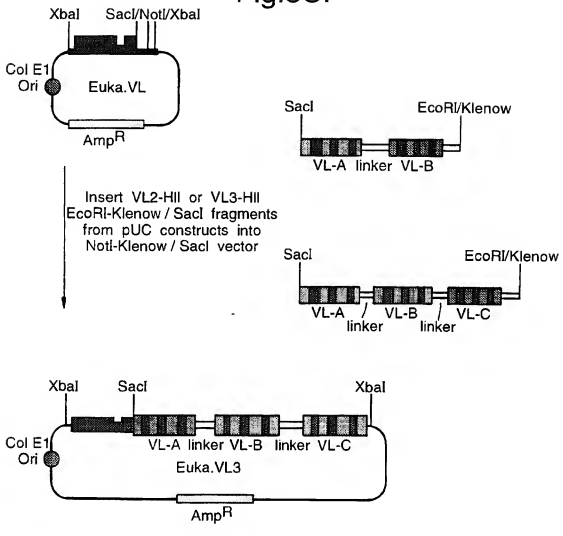

XbaI
SacI/NotI/XbaI
Col E1 Ori
Euka.VL
AmpR
SacI
EcoRI/Klenow
VL-A linker VL-B
Insert VL2-HII or VL3-HII EcoRI-Klenow / SacI fragments from pUC constructs into NotI-Klenow / SacI vector
SacI
EcoRI/Klenow
VL-A
linker
VL-B
linker
VL-C
XbaI
SacI
XbaI
Col E1 Ori
VL-A linker VL-B linker VL-C
Euka.VL3
AmpR
Excise XbaI / XbaI fragments from Euka constructs and insert into XbaI opened pSV.51 vector
pSV.N/pSV.P/pSV.S/pSV.V/pSV.W

Fig.9.

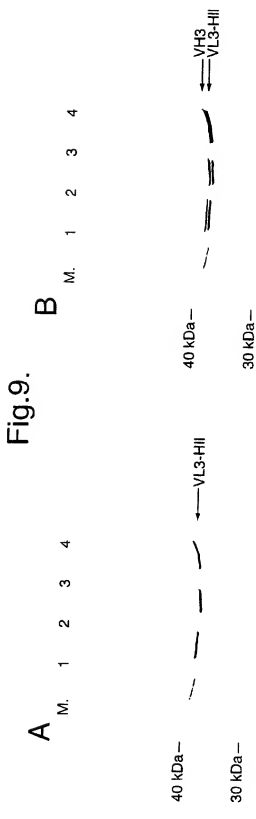
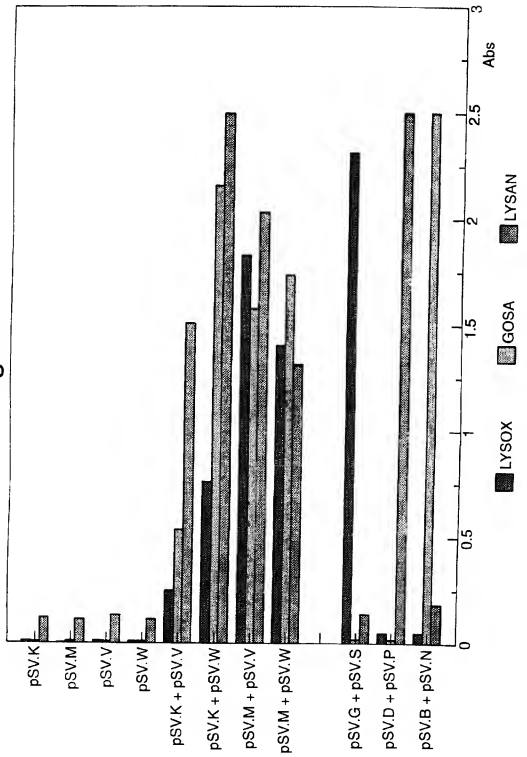


Fig.10.


pSV.K
pSV.M
pSV.V
pSV.W
pSV.K + pSV.V
pSV.K + pSV.W
pSV.M + pSV.V
pSV.M + pSV.W
pSV.G + pSV.S
pSV.D + pSV.P
pSV.B + pSV.N
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0.5
1
1.5
2
2.5
3
Abs
LYSOX
GOSA
LYSAN

Fig.11.

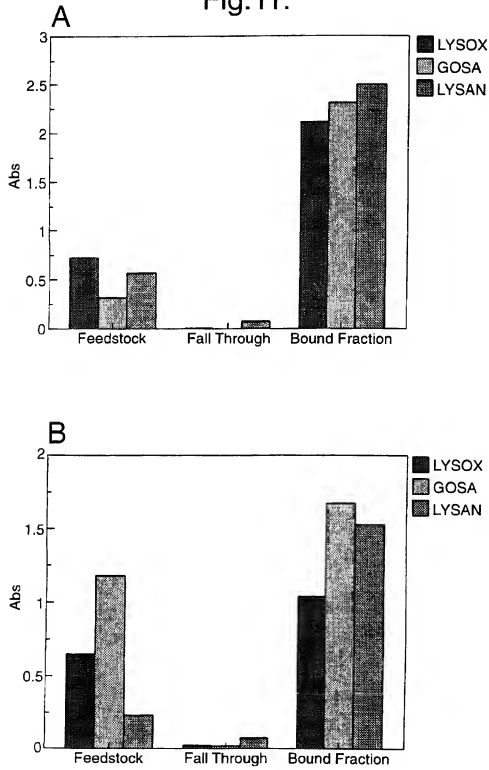
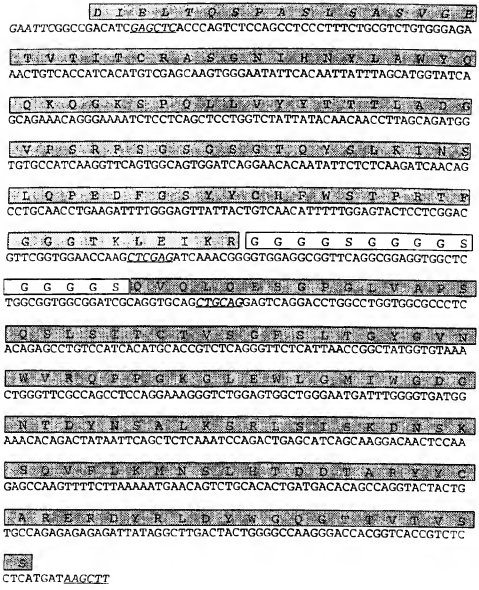

A
3
2.5
2
1.5
1
0.5
0
Abs
LYSOX
GOSA
LYSAN
Feedstock
Fall Through
Bound Fraction
B
2
1.5
1
0.5
0
Abs
LYSOX
GOSA
LYSAN
Feedstock
Fall Through
Bound Fraction

Fig.12.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GCAGAAACAGGGAAAATCTCCTCAGCTCCTGGTCTATTATACAACAACCTTAGCAGATGG
VL Lys
V P S R F S G S G S G T Q Y S L K I N S
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L Q P E D F G S Y Y C H F W S T P R T F
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G G G T K L E I K R G G G G S G G G G S
GTTCGGTGGAACCAAGCTCGAGATCAAACGGGGTGGAGGCGGTTCAGGCGGAGGTGGCTC
Linker
G G G G S Q V Q L Q E S G P G L V A P S
TGGCGGTGGCGGATCGCAGGTGCAGCTGCAGGAGTCAGGACCTGGCCTGGTGGCGCCCTC
Q S L S I T C T V S G F S L T G Y G V N
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W V R Q P P G K G L E W L G M I W G D G
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N T D Y N S A L K S R L S I S K D N S K
VH Lys AAACACAGACTATAATTCAGCTCTCAAATCCAGACTGAGCATCAGCAAGGACAACTCCAA
S Q V F L K M N S L H T D D T A R Y Y C
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A R E R D Y R L D Y W G Q G T T V T V S
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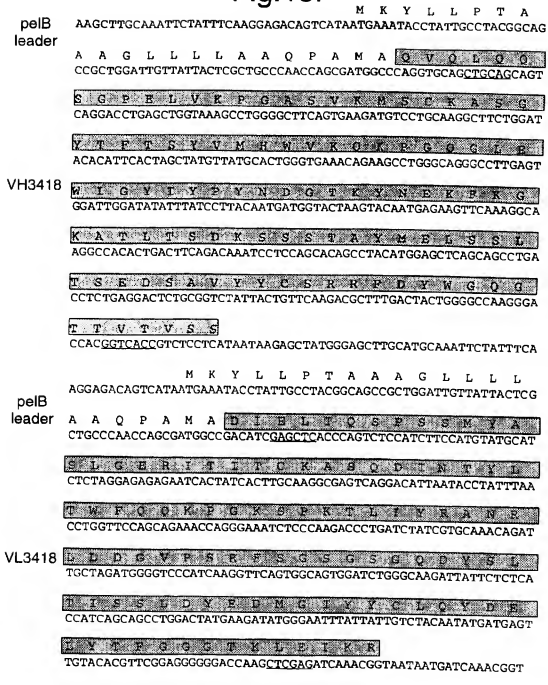
S
CTCATGATAAGCTT

Fig.13.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S G P E L V K P G A S V K M S C K A S G
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VH3418
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T S E D S A V Y Y C S R K P D Y W G Q G
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T T V T V S S
CCACGGTCACCGTCTCCTCATAATAAGAGCTATGGGAGCTTGCATGCAAATTCTATTTCA
M K Y L L P T A A A G L L L L
AGGAGACAGTCATAATGAAATACCTATTGCCTACGGCAGCCGCTGGATTGTTATTACTCG
pelB leader
A A Q P A M A D I E L T Q S P S S M Y A
CTGCCCAACCAGCGATGGCCGACATCGAGCTCACCCAGTCTCCATCTTCCATGTATGCAT
S L G E R I T I T C K A S Q D I N T Y L
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VL3418
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T I S S L D Y E D M G I Y Y C L Q Y D E
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T Y T F G G G T K L E I K R
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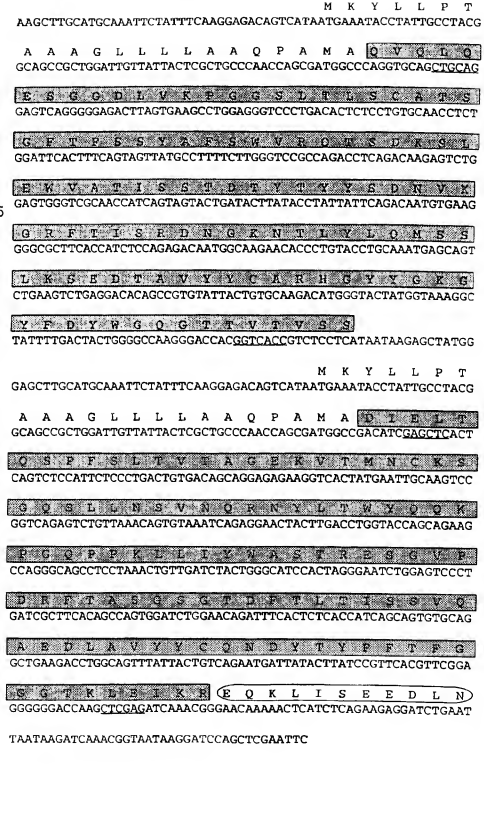
Fig.14.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E S G G D L V K P G G S L T L S C A T S
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E W V A T I S S T D T Y T Y Y S D N V K
VH4715
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L K S E D T A V Y Y C A R H G Y Y G K G
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Y F D Y R G Q G T T V T V S S
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M K Y L L P T
pelB leader
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A A A G L L L L A A Q P A M A D I E L T
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VL4715
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Myc-tag
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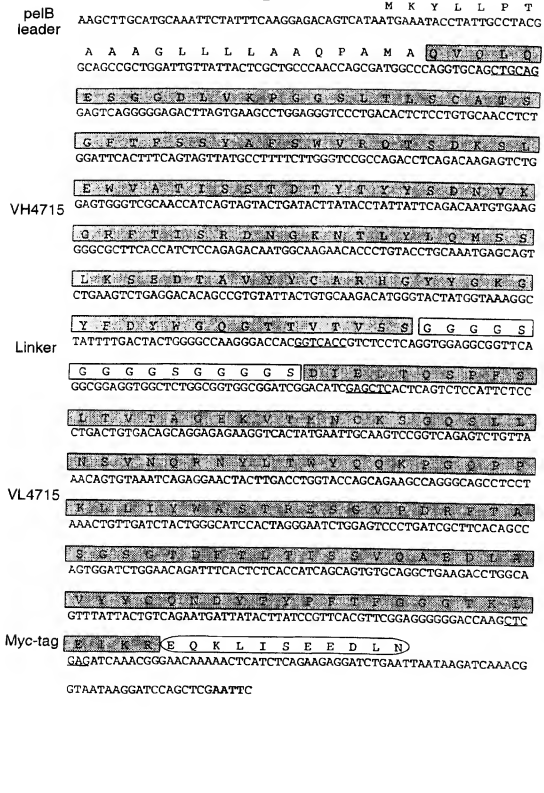
Fig.15.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E S G G D L V K P G G S L T L S C A T S
GAGTCAGGGGGAGACTTAGTGAAGCCTGGAGGGTCCCTGACACTCTCCTGTGCAACCTCT
G F T F S S Y A F S W V R Q T S D K S L
GGATTCACTTTCAGTAGTTATGCCTTTTCTTGGGTCCGCCAGACCTCAGACAAGAGTCTG
E W V A T I S S T D T Y T Y Y S D N V K
VH4715
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G R F T I S R D N G K N T L Y L Q M S S
GGGCGCTTCACCATCTCCAGAGACAATGGCAAGAACACCCTGTACCTGCAAATGAGCAGT
L K S E D T A V Y Y C A R H G Y Y G K G
CTGAAGTCTGAGGACACAGCCGTGTATTACTGTGCAAGACATGGGTACTATGGTAAAGGC
Y F D Y W G Q G T T V T V S S G G G G S
Linker
TATTTTGACTACTGGGGCCAAGGGACCACGGTCACCGTCTCCTCAGGTGGAGGCGGTTCA
G G G G S G G G G S D I E L T Q S P F S
GGCGGAGGTGGCTCTGGCGGTGGCGGATCGGACATCGAGCTCACTCAGTCTCCATTCTCC
L T V T A G E K V T K N C K S D Q S L L
CTGACTGTGACAGCAGGAGAGAAGGTCACTATGAATTGCAAGTCCGGTCAGAGTCTGTTA
N S V N Q R N Y L T W Y Q Q K P G Q P P
AACAGTGTAAATCAGAGGAACTACTTGACCTGGTACCAGCAGAAGCCAGGGCAGCCTCCT
VL4715
K L L I Y W A S T R E S G V P D R F T A
AAACTGTTGATCTACTGGGCATCCACTAGGGAATCTGGAGTCCCTGATCGCTTCACAGCC
S G S G T D F T L T I S S V Q A E D L A
AGTGGATCTGGAACAGATTTCACTCTCACCATCAGCAGTGTGCAGGCTGAAGACCTGGCA
V Y Y C Q N D Y S Y P F T F G G G T K L
GTTTATTACTGTCAGAATGATTATACTTATCCGTTCACGTTCGGAGGGGGGACCAAGCTC
Myc-tag
E I K R E Q K L I S E E D L N
GAGATCAAACGGGAACAAAAACTCATCTCAGAAGAGGATCTGAATTAATAAGATCAAACG
GTAATAAGGATCCAGCTCGAATTC

Fig.16A.

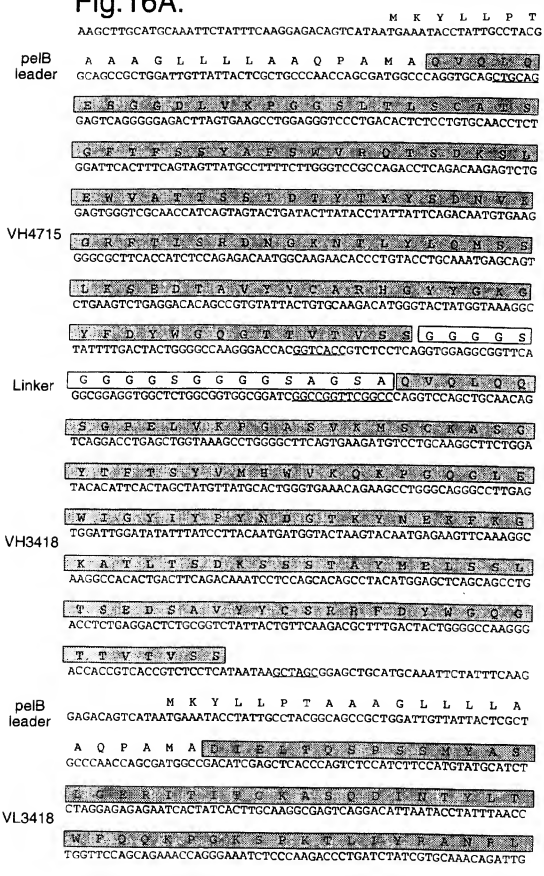
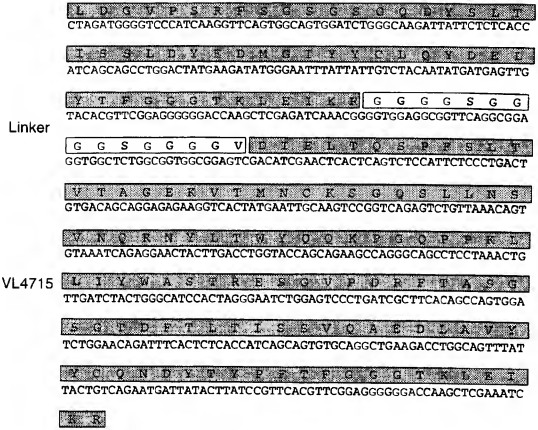

M K Y L L P T
AAGCTTGCATGCAAATTCTATTTCAAGGAGACAGTCATAATGAAATACCTATTGCCTACG
pelB leader
A A A G L L L L A A Q P A M A Q V Q L Q
GCAGCCGCTGGATTGTTATTACTCGCTGCCCAACCAGCGATGGCCCAGGTGCAGCTGCAG
E S G G D L V K P G G S L T L S C A T S
GAGTCAGGGGGAGACTTAGTGAAGCCTGGAGGGTCCCTGACACTCTCCTGTGCAACCTCT
G F T F S S Y A F S W V R Q T S D K S L
GGATTCACTTTCAGTAGTTATGCCTTTTCTTGGGTCCGCCAGACCTCAGACAAGAGTCTG
E W V A T I S S G G T Y T Y Y R D N V K
GAGTGGGTCGCAACCATCAGTAGTACTGATACTTATACCTATTATTCAGACAATGTGAAG
VH4715
G R F T I S R D N A K N T L Y L Q M D S
GGGCGCTTCACCATCTCCAGAGACAATGGCAAGAACACCCTGTACCTGCAAATGAGCAGT
L K S E D T A V Y Y C A R H G Y Y G K G
CTGAAGTCTGAGGACACAGCCGTGTATTACTGTGCAAGACATGGGTACTATGGTAAAGGC
Y F D Y W G Q G T T V T V S S G G G G S
TATTTTGACTACTGGGGCCAAGGGACCACGGTCACCGTCTCCTCAGGTGGAGGCGGTTCA
Linker
G G G G S G G G G S A G S A Q V Q L Q Q
GGCGGAGGTGGCTCTGGCGGTGGCGGATCGGCCGGTTCGGCCCAGGTCCAGCTGCAACAG
S G P E L V K P G A S V K M S C K A S G
TCAGGACCTGAGCTGGTAAAGCCTGGGGCTTCAGTGAAGATGTCCTGCAAGGCTTCTGGA
Y T F T S Y V M H W V K Q K P G Q G L E
TACACATTCACTAGCTATGTTATGCACTGGGTGAAACAGAAGCCTGGGCAGGGCCTTGAG
W I G Y I Y P Y N D G T K Y N E K F K G
VH3418
TGGATTGGATATATTTATCCTTACAATGATGGTACTAAGTACAATGAGAAGTTCAAAGGC
K A T L T S D K S S S T A Y M E L S S L
AAGGCCACACTGACTTCAGACAAATCCTCCAGCACAGCCTACATGGAGCTCAGCAGCCTG
T S E D S A V Y Y C S R R F D Y W G Q G
ACCTCTGAGGACTCTGCGGTCTATTACTGTTCAAGACGCTTTGACTACTGGGGCCAAGGG
T T V T V S S
ACCACCGTCACCGTCTCCTCATAATAAGCTAGCGGAGCTGCATGCAAATTCTATTTCAAG
M K Y L L P T A A A G L L L L A
pelB leader
GAGACAGTCATAATGAAATACCTATTGCCTACGGCAGCCGCTGGATTGTTATTACTCGCT
A Q P A M A D I E L T Q S P S S M Y A S
GCCCAACCAGCGATGGCCGACATCGAGCTCACCCAGTCTCCATCTTCCATGTATGCATCT
L G E R I T I N C K A S Q D I N T Y L T
VL3418
CTAGGAGAGAGAATCACTATCACTTGCAAGGCGAGTCAGGACATTAATACCTATTTAACC
W F Q Q K P G K S P K T L I Y R A N R L
TGGTTCCAGCAGAAACCAGGGAAATCTCCCAAGACCCTGATCTATCGTGCAAACAGATTG

Fig.16B.


L D G V P S R F S G S G S G T D Y S L T
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I S S L D V E D M G I Y Y C L Q Y D E L
ATCAGCAGCCTGGACTATGAAGATATGGGAATTTATTATTGTCTACAATATGATGAGTTG
Y T F G G G T K L E I K R G G G G S G G
TACACGTTCGGAGGGGGGACCAAGCTCGAGATCAAACGGGGTGGAGGCGGTTCAGGCGGA
Linker
G G S G G G G V D I E L T Q S P F S L T
GGTGGCTCTGGCGGTGGCGGAGTCGACATCGAACTCACTCAGTCTCCATTCTCCCTGACT
V T A G E K V T M N C K S S Q S L L N S
GTGACAGCAGGAGAGAAGGTCACTATGAATTGCAAGTCCGGTCAGAGTCTGTTAAACAGT
V N Q K N Y L T W Y Q Q K P G Q P P K L
GTAAATCAGAGGAACTACTTGACCTGGTACCAGCAGAAGCCAGGGCAGCCTCCTAAACTG
VL4715
L I Y W A S T R E S G V P D R F T A S G
TTGATCTACTGGGCATCCACTAGGGAATCTGGAGTCCCTGATCGCTTCACAGCCAGTGGA
S G T D F T L T I S S V Q A E D L A V Y
TCTGGAACAGATTTCACTCTCACCATCAGCAGTGTGCAGGCTGAAGACCTGGCAGTTTAT
Y C Q N D Y T Y P F T F G S G T K L E I
TACTGTCAGAATGATTATACTTATCCGTTCACGTTCGGAGGGGGGACCAAGCTCGAAATC
K S
AAACGGTAATAAGCGGCCGCGAATTC

Fig.16C.

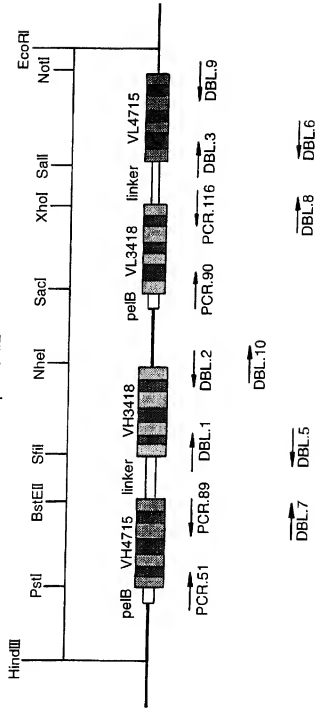

pGOSA.E
HindIII
PstI
BstEII
SfiI
NheI
SacI
XhoI
SalI
NotI
EcoRI
pelB
VH4715
linker
VH3418
pelB
VL3418
linker
VL4715
PCR.51
PCR.89
DBL.1
DBL.2
PCR.90
PCR.116
DBL.3
DBL.9
DBL.10
DBL.7
DBL.5
DBL.8
DBL.6

Fig.17.

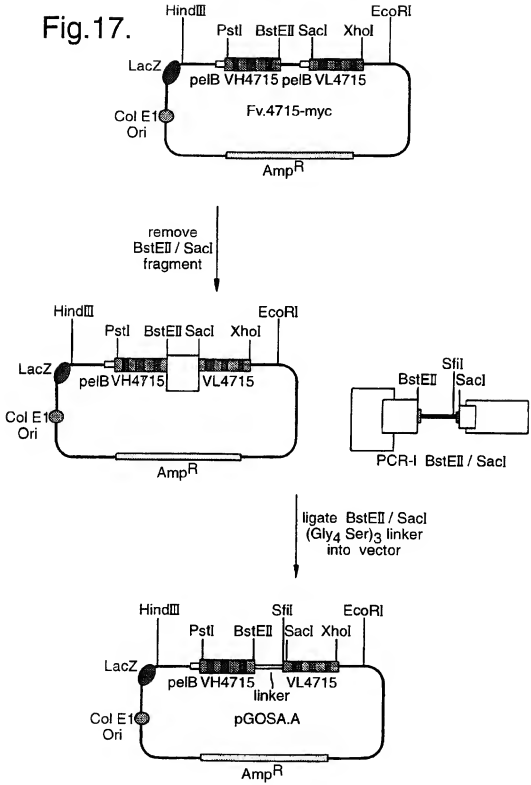

HindIII
PstI
BstEII
SacI
XhoI
EcoRI
LacZ
pelB VH4715
pelB VL4715
Fv.4715-myc
Col E1 Ori
AmpR
remove BstEII / SacI fragment
VL4715
SfiI
PCR-I
ligate BstEII / SacI (Gly4 Ser)3 linker into vector
linker
pGOSA.A

Fig.18.

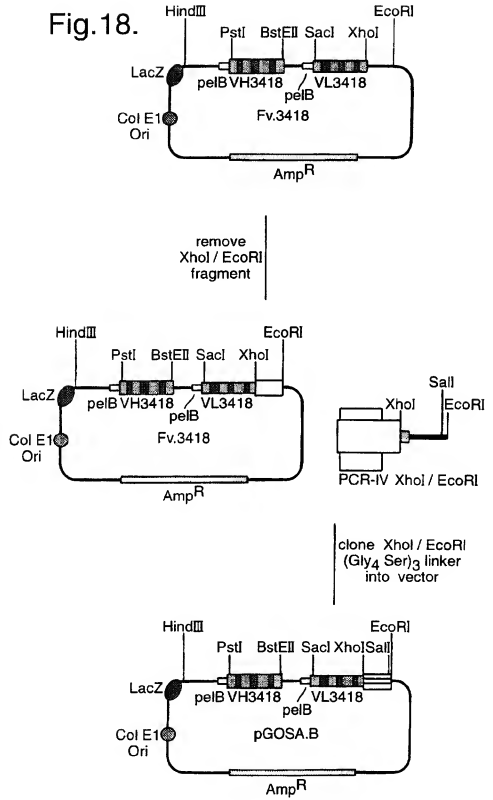

HindIII
EcoRI
PstI
BstEII
SacI
XhoI
LacZ
pelB VH3418
pelB
VL3418
Col E1 Ori
Fv.3418
AmpR
remove XhoI / EcoRI fragment
SalI
XhoI
EcoRI
PCR-IV XhoI / EcoRI
clone XhoI / EcoRI (Gly4 Ser)3 linker into vector
pGOSA.B

Fig.19.

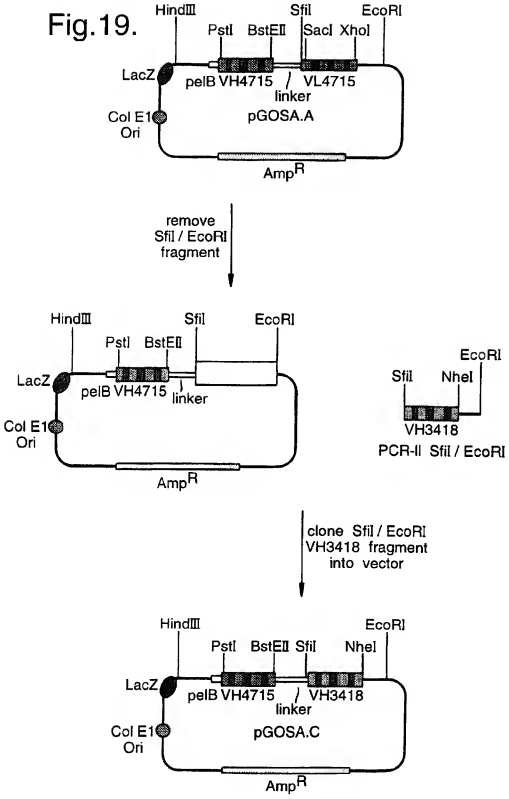

HindIII
SfiI
EcoRI
PstI
BstEII
SacI
XhoI
LacZ
pelB VH4715
/
VL4715
linker
Col E1
Ori
pGOSA.A
AmpR
remove
SfiI / EcoRI
fragment
HindIII
SfiI
EcoRI
PstI
BstEII
LacZ
pelB VH4715
linker
Col E1
Ori
AmpR
EcoRI
SfiI
NheI
VH3418
PCR-II SfiI / EcoRI
clone SfiI / EcoRI
VH3418 fragment
into vector
HindIII
EcoRI
PstI
BstEII
SfiI
NheI
LacZ
pelB VH4715
/
VH3418
linker
Col E1
Ori
pGOSA.C
AmpR

Fig.20.

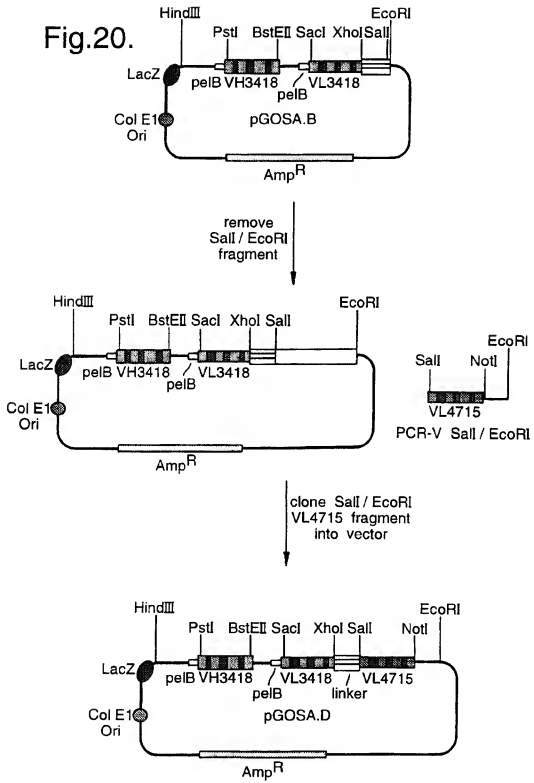

HindIII
PstI
BstEII
SacI
XhoI
SalI
EcoRI
LacZ
pelB
VH3418
pelB
VL3418
Col E1 Ori
pGOSA.B
AmpR
remove SalI / EcoRI fragment
NotI
VL4715
PCR-V SalI / EcoRI
clone SalI / EcoRI VL4715 fragment into vector
linker
pGOSA.D

Fig.21.

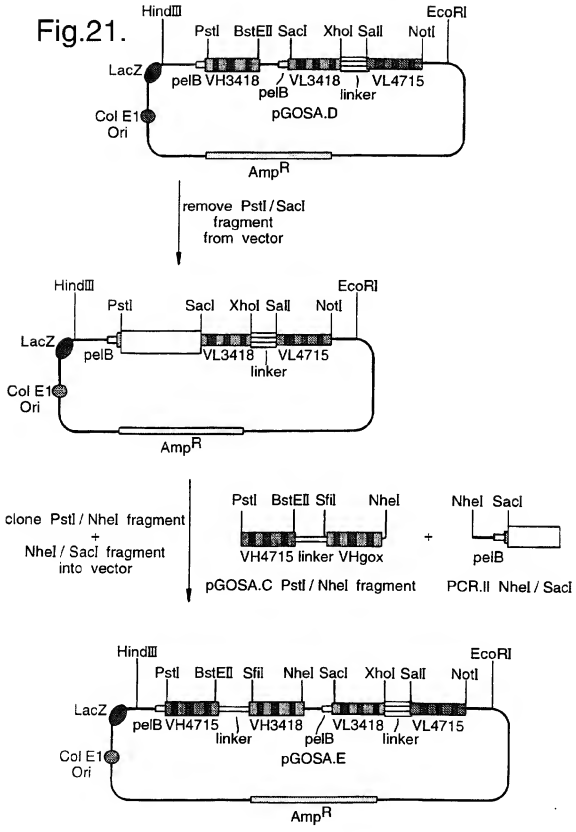

HindIII
PstI
BstEII
SacI
XhoI
SalI
NotI
EcoRI
LacZ
pelB
VH3418
VL3418
VL4715
linker
Col E1 Ori
pGOSA.D
AmpR
remove PstI / SacI fragment from vector
clone PstI / NheI fragment + NheI / SacI fragment into vector
SfiI
NheI
VH4715 linker VHgox
pGOSA.C PstI / NheI fragment
PCR.II NheI / SacI
pGOSA.E

Fig.22.

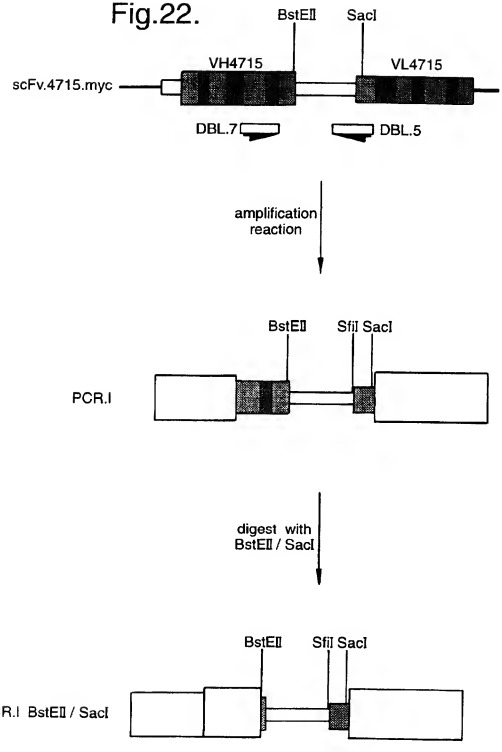

BstEII
SacI
VH4715
VL4715
scFv.4715.myc
DBL.7
DBL.5
amplification reaction
BstEII
SfiI SacI
PCR.I
digest with BstEII / SacI
BstEII
SfiI SacI
PCR.I BstEII / SacI

Fig.23.

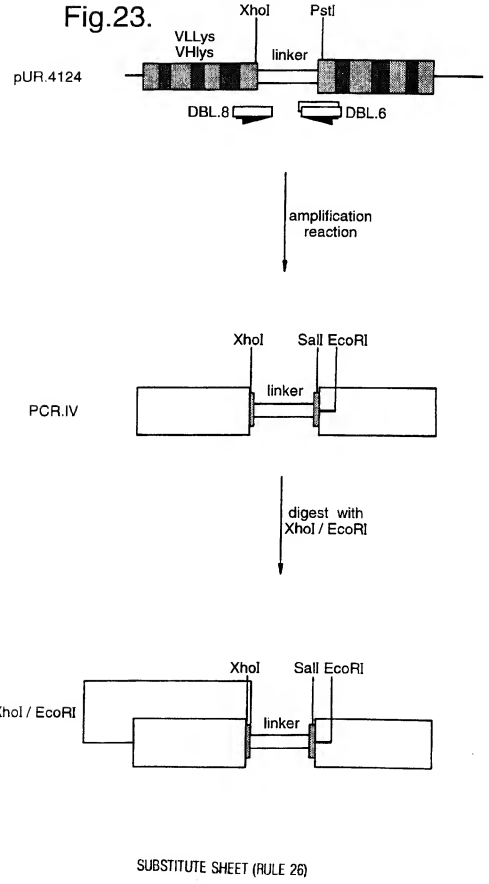

XhoI
PstI
VLLys
VHlys
linker
pUR.4124
DBL.8
DBL.6
amplification reaction
XhoI
SalI EcoRI
linker
PCR.IV
digest with XhoI / EcoRI
XhoI
SalI EcoRI
PCR.IV XhoI / EcoRI
linker

Fig.24.

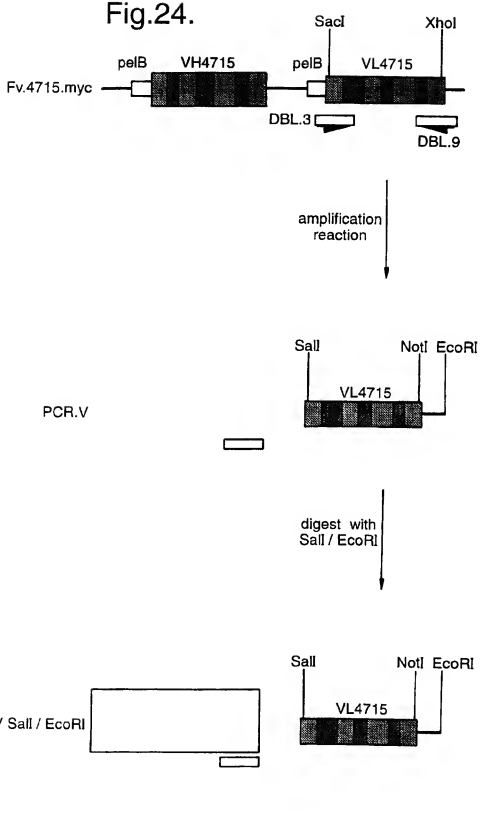

SacI
XhoI
pelB
VH4715
pelB
VL4715
Fv.4715.myc
DBL.3
DBL.9
amplification reaction
SalI
NotI
EcoRI
VL4715
PCR.V
digest with SalI / EcoRI
SalI
NotI
EcoRI
VL4715
PCR.V SalI / EcoRI

Fig.25.

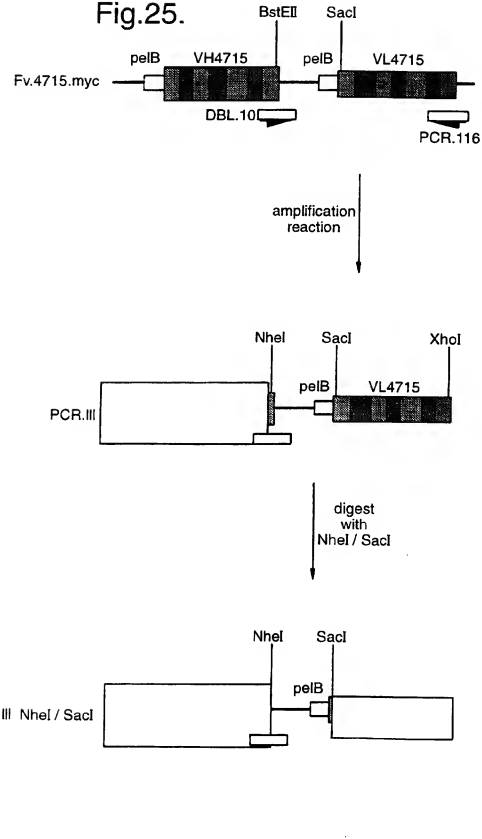
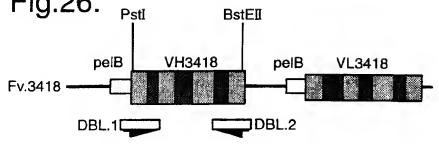

BstEII
SacI
pelB
VH4715
pelB
VL4715
Fv.4715.myc
DBL.10
PCR.116
amplification reaction
NheI
SacI
XhoI
pelB
VL4715
PCR.III
digest with NheI / SacI
NheI
SacI
pelB
PCR.III NheI / SacI

Fig.26.


PstI
BstEII
pelB
VH3418
pelB
VL3418
Fv.3418
DBL.1
DBL.2
amplification reaction
SfiI
NheI
EcoRI
VH3418
PCR.II
digest with SfiI / EcoRI
SfiI
NheI
EcoRI
VH3418
PCR.II SfiI / EcoRI

SEQ ID NO.		
5	PCR.51	: 5' AGG T(C/G)(A/C) A(C/A)C TGC AG(C/G) AGT C(A/T)G G 3'
6	PCR.89	: 5' TGA GGA GAC GGT GAC CGT GGT CCC TTG GCC CC 3'
7	PCR.90	: 5' GAC ATT GAG CTC ACC CAG TCT CCA 3'
8	PCR.116	: 5' GTT AGA TCT CGA GCT TGG TCC C 3'
9	DBL.1	: 5' CAG GAT CCG GCC GGT TCG GCC CAG GTC CAG CTG CAA CAG TCA GGA '3
10	DBL.2	: 5' CTA CAT GAA TTC GCT AGC TTA TTA TGA GGA GAC GGT GAC GGT GGT CCC TTG GC '3
11	DBL.3	: 5' ATT GGA GTC GAC ATC GAA CTC ACT CAG TCT CCA TTC TCC 3'
12	DBL.4	: 5' CGA ATT CGG ATC CCC GTT TGA TTT CGA GCT TGG TCC '3
13	DBL.5	: 5' GAG CGC GAG CTC GGC CGA ACC GGC CGA TCC GCC ACC GCC AGA GCC '3
14	DBL.6	: 5' AAT GTC GAA TTC GTC GAC TCC GCC ACC GCC AGA GCC '3
15	Euka.1	: 5' AGC TTC TAG ACC ACC ATG GAA AAC TGC AGA GCT CAA AAG CTA GCG CGG CGG CTC TAG '3
16	Euka.2	: 5' AAT TCT AGA GCG GCC GCG CTA GCT TTT GAG CTC TGC AGT TTT CCA TGG TGG TCT AGA '3
17	ML.1	: 5' ACG GGT GAG CTC GAT GTC GGA GTG GAC ACC TGT GGA GAG A '3
18	ML.2	: 5' GGA AAC AGC TAT GAC CAT GAT TAC '3
19	DBL.7	: 5' CAC CAT CTC CAG AGA CAA TGG CAA G 3'
20	DBL.8	: 5' ACC AAG CTC GAG ATC AAA CGG GG 3'
21	DBL.9	: 5' TGA AGT GAA TTC GCG GCC GCT TAT TAC CGT TTG ATT TCG AGC TTG GTC CC 3'
22	DBL.10	: 5' TAA TAA GCT AGC GGA GCT GCA TGG AAA TTC TAT TTC 3'

Table 1

Code		Expressed Antibody fragment
pSV.B	:	VH4715-VH3418
pSV.D	:	VH4715-VHlys
pSV.G	:	VH3418-VHlys
pSV.K	:	VH4715-VHlys-VH3418
pSV.M	:	VHlys-VH4715-VH3418
pSV.N	:	VL3418-VL4715.2t
pSV.P	:	VLlys-VL4715.2t
pSV.S	:	VLlys-VL3418.2t
pSV.V	:	VLlys-VL4715-VL3418.2t
pSV.W	:	VL3418-VLlys-VL4715.2t

Table 2

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 97/01609

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/13 C07K16/46 C07K16/00 C12N5/10 A61K39/395
G01N33/577

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N C07K A61K G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 93 11161 A (ENZON, INC.) 10 June 1993 cited in the application see page 22, line 1 - line 10 see claims ---	1-15
A	WO 94 09131 A (SCOTGEN LTD.) 28 April 1994 cited in the application see claims see figures --- -/--	1-15

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
14 August 1997	10.09.97
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax (+ 31-70) 340-3016	Authorized officer Nooij, F

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE USA, vol. 90, no. 14, 15 July 1993, WASHINGTON, DC, USA, pages 6444-6448, XP002014058 P. HOLLIGER ET AL.: ""Diabodies": Small bivalent and bispecific antibody fragments." see the whole document	1-15
A	WO 94 13806 A (THE DOW CHEMICAL COMPANY) 23 June 1994 see figure 1	1-15
A	WO 94 13804 A (CAMBRIDGE ANTIBODY TECHNOLOGY LTD. ET AL.) 23 June 1994 see page 31, line 10 - line 12 see figure 1	1-15
T	WO 97 14719 A (UNILEVER) 24 April 1997 see the whole document	1-15

INTERNATIONAL SEARCH REPORT

Int[illegible]ional application No.
PCT/EP 97/01609

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 14
because they relate to subject matter not required to be searched by this Authority, namely:

Remark: Although claim(s) 14
is(are) directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Internati Application No
PCT/EP 97/01609

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9311161 A	10-06-93	AU 3178993 A	28-06-93
		CA 2122732 A	10-06-93
		EP 0617706 A	05-10-94
		JP 7501451 T	16-02-95
WO 9409131 A	28-04-94	AU 5283793 A	09-05-94
		CA 2146854 A	28-04-94
		GB 2286189 A	09-08-95
		JP 8505761 T	25-06-96
WO 9413806 A	23-06-94	AU 5747794 A	04-07-94
		CA 2117477 A	23-06-94
		EP 0628078 A	14-12-94
		JP 7503622 T	20-04-95
WO 9413804 A	23-06-94	AU 5654894 A	04-07-94
		CA 2150262 A	23-06-94
		EP 0672142 A	20-09-95
		JP 8504100 T	07-05-96
		AU 7621494 A	10-04-95
		CA 2169620 A	30-03-95
		EP 0720624 A	10-07-96
		WO 9508577 A	30-03-95
WO 9714719 A	24-04-97	AU 6873396 A	07-05-97